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NATURAL VARIATION IN LEARNING ABILITY IN DROSOPHILA MELANOGASTER

NÉPOUX Virginie

NÉPOUX Virginie, 2011, NATURAL VARIATION IN LEARNING ABILITY IN DROSOPHILA MELANOGASTER

Originally published at : Thesis, University of Lausanne

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UNIL | Université de Lausanne
Faculté de biologie et de médecine

Département Écologie et Évolution

NATURAL VARIATION IN LEARNING ABILITY IN *DROSOPHILA MELANOGASTER*

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

Virginie NÉPOUX

Master de l'Université de Paris XIII Villetaneuse

Jury

Prof. Nicolas Mermod, Président
Prof. Tadeusz Kawecki, Directeur de thèse

Prof. Michael Ritchie, Expert
Dr. Frédéric Mery, Expert

Lausanne 2011

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président	Monsieur	Prof. Nicolas **Mermod**
Directeur de thèse	Monsieur	Prof. Tadeusz **Kawecki**
Experts	Monsieur	Prof. Michael **Ritchie**
	Monsieur	Dr Frédéric **Mery**

le Conseil de Faculté autorise l'impression de la thèse de

Madame Virginie Nepoux

Master de l'Université de Paris XIII

intitulée

Natural variation in learning ability in *Drosophila melanogaster*

Lausanne, le 22 décembre 2011

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Nicolas Mermod

Natural genetic variation of learning ability in *Drosophila*

Contents

Summary

Learning is the ability of an organism to adapt to the changes of its environment in response to its past experience. It is a widespread ability in the animal kingdom, but its evolutionary aspects are poorly known. Learning ability is supposedly advantageous under some conditions, when environmental conditions are not too stable - because in this case there is no need to learn to predict any event in the environment - and not changing too fast - otherwise environmental cues cannot be used because they are not reliable. Nevertheless, learning ability is also known to be costly in terms of energy needed for neuronal synthesis, memory formation, initial mistakes. During my PhD, I focused on the study of genetic variability of learning ability in natural populations. Genetic variability is the basis on which natural selection and genetic drift can act. How does learning ability vary in nature? What are the roles of additive genetic variation or maternal effects in this variation? Is it involved in evolutionary trade-offs with other fitness-related traits?

I investigated a natural population of fruit fly, *Drosophila melanogaster,* as a model organism. Its learning ability is easy to measure with associative memory tests. I used two research tools: multiple inbred and isofemale lines derived from a natural population as a representative sample. My work was divided into three parts.

First, I investigated the effects of inbreeding on aversive learning (avoidance of an odor previously associated with mechanical shock). While the inbred lines consistently showed reduced egg-to-adult viability by 28 %, the effects of inbreeding on learning performance was 18 % and varied among assays, with a trend to be most pronounced for intermediate conditioning intensity. Variation among inbred lines indicates that ample genetic variance for learning was segregating in the base population, and suggests that the inbreeding depression observed in learning performance was mostly due to dominance rather than overdominance. Across the inbred lines, learning performance was positively correlated with the egg-to-adult viability. This positive genetic correlation contradicts previous studies which observed a trade-off between learning ability and lifespan or larval competitive ability. It suggests that much of the genetic variation for learning is due to pleiotropic effects of genes affecting other functions related to survival. Together with the overall mild effects of inbreeding on learning performance, this suggests that genetic variation specifically affecting learning is either very low, or is due to alleles with mostly additive (semi-dominant) effects. It also suggests that alleles reducing learning performance are on average partially recessive, because their effect does not appear in the outbred base population. Moreover, overdominance seems unlikely as major cause of the inbreeding depression, because even if the overall mean of the inbred line is smaller than the outbred base population, some of the inbred lines show the same learning score as the outbred base population. If overdominance played an important part in inbreeding depression, then all the homozygous lines should show lower learning ability than

outbred base population.

In the second part of my project, I sampled the same natural population again and derived isofemale lines (F=0.25) which are less adapted to laboratory conditions and therefore are more representative of the variance of the natural population. They also showed some genetic variability for learning, and for three other fitness-related traits possibly related with learning: resistance to bacterial infection, egg-to-adult viability and developmental time. Nevertheless, the genetic variance of learning ability did not appear to be smaller than the variance of the other traits. The positive correlation previously observed between learning ability and egg-to-adult viability did not appear in isofemale lines (nor a negative correlation). It suggests that there was still genetic variability within isofemale lines and that they did not fix the highly deleterious pleiotropic alleles possibly responsible for the previous correlation.

In order to investigate the relative amount of nuclear (additive and non-additive effects) and extra-nuclear (maternal and paternal effect) components of variance in learning ability and other fitness-related traits among the inbred lines tested in part one, I performed a diallel cross between them. The nuclear additive genetic variance was higher than other components for learning ability and survival to learning ability, but in contrast, maternal effects were more variable than other effects for developmental traits. This suggests that maternal effects, which reflects effects from mitochondrial DNA, epigenetic effects, or the amount of nutrients that are invested by the mother in the egg, are more important in the early stage of life, and less at the adult stage. There was no additive genetic correlation between learning ability and other traits, indicating that the correlation between learning ability and egg-to-adult viability observed in the first pat of my project was mostly due to recessive genes.

Finally, my results showed that learning ability is genetically variable. The diallel experiment showed additive genetic variance was the most important component of the total variance. Moreover, every inbred or isofemale line showed some learning ability. This suggested that alleles impairing learning ability are eliminated by selection, and therefore that learning ability is under strong selection in natural populations of *Drosophila*. My results cannot alone explain the maintenance of the observed genetic variation. Even if I cannot eliminate the hypothesis of pleiotropy between learning ability and the other fitness-related traits I measured, there is no evidence for any trade-off between these traits and learning ability. This contradicts what has been observed between learning ability and other traits like lifespan and larval competitivity.

Résumé en français

L'apprentissage représente la capacité d'un organisme à s'adapter aux changement de son environnement au cours de sa vie, en réponse à son expérience passée. C'est une capacité très répandue dans le règne animal, y compris pour les animaux les plus petits et les plus simples, mais les aspects évolutifs de l'apprentissage sont encore mal connus.

L'apprentissage est supposé avantageux dans certaines conditions, quand l'environnement n'est ni trop stable – dans ce cas, il n'y a rien à apprendre – ni trop variable – dans ce cas, les indices sur lesquels se reposer changent trop vite pour apprendre. D'un autre côté, l'apprentissage a aussi des coûts, en terme de synthèse neuronale, pour la formation de la mémoire, ou de coûts d'erreur initiale d'apprentissage. Pendant ma thèse, j'ai étudié la variabilité génétique naturelle des capacités d'apprentissage. Comment varient les capacités d'apprentissage dans la nature ? Quelle est la part de variation additive, l'impact des effets maternel ? Est-ce que l'apprentissage est impliqué dans des interactions, de type compromis évolutifs, avec d'autres traits liés à la fitness ?

Afin de répondre à ces questions, je me suis intéressée à la mouche du vinaigre, ou drosophile, un organisme modèle. Ses capacités d'apprentissage sont facile à étudier avec un test de mémoire reposant sur l'association entre un choc mécanique et une odeur. Pour étudier ses capacités naturelles, j'ai dérivé de types de lignées d'une population naturelle: des lignées consanguines et des lignées isofemelles.

Dans une première partie, je me suis intéressée aux effets de la consanguinité sur les capacités d'apprentissage, qui sont peu connues. Alors que les lignées consanguines ont montré une réduction de 28% de leur viabilité (proportion d'adultes émergeants d'un nombre d'œufs donnés), leurs capacités d'apprentissage n'ont été réduites que de 18%, la plus forte diminution étant obtenue pour un conditionnement modéré. En outre, j'ai également observé que les capacités d'apprentissage était positivement corrélée à la viabilité entre les lignées. Cette corrélation est surprenante car elle est en contradiction avec les résultats obtenus par d'autres études, qui montrent l'existence de compromis évolutifs entre les capacités d'apprentissage et d'autres traits comme le vieillissement ou la compétitivité larvaire. Elle suggère que la variation génétique des capacités d'apprentissage est due aux effets pleiotropes de gènes récessifs affectant d'autres fonctions liées à la survie. Ces résultats indiquent que la variation pour les capacités d'apprentissage est réduite comparée à celle d'autres traits ou est due à des allèles principalement récessifs. L'hypothèse de superdominance semble peu vraisemblable, car certaines des lignées consanguines ont obtenu des scores d'apprentissage égaux à ceux de la population non consanguine, alors qu'en cas de superdominance, elles auraient toutes dû obtenir des scores inférieurs.

Dans la deuxième partie de mon projet, j'ai mesuré les capacités d'apprentissage de lignées isofemelles issues de la même population initiale que les lignées consanguines. Ces lignées

sont issues chacune d'un seul couple, ce qui leur donne un taux d'hétérozygosité supérieur et évite l'élimination de lignées par fixation d'allèles délétères rares. Elles sont ainsi plus représentatives de la variabilité naturelle. Leur variabilité génétique est significative pour les capacités d'apprentissage, et trois traits liés à la fois à la fitness et à l'apprentissage: la viabilité, la résistance à l'infection bactérienne et la vitesse de développement. Cependant, la variabilité des capacités d'apprentissage n'apparaît cette fois pas inférieure à celle des autres traits et aucune corrélation n'est constatée entre les capacité d'apprentissage et les autres traits. Ceci suggère que la corrélation observée auparavant était surtout due à la fixation d'allèles récessifs délétères également responsables de la dépression de consanguinité.

Durant la troisième partie de mon projet, je me suis penchée sur la décomposition de la variance observée entre les lignées consanguines observée en partie 1. Quatre composants ont été examinés: la variance due à des effets nucléaires (additifs et non additifs), et due à des effets parentaux (maternels et paternels). J'ai réalisé un croisement diallèle de toutes les lignées. La variance additive nucléaire s'est révélée supérieure aux autres composants pour les capacités d'apprentissage et la résistance à l'infection bactérienne. Par contre, les effets maternels étaient plus importants que les autres composants pour les traits développementaux (viabilité et vitesse de développement). Ceci suggère que les effets maternels, dus à l'ADN mitochondrial, à l'épistasie ou à la quantité de nutriments investis dans l'œuf par la mère, sont plus importants dans les premiers stades de développement et que leur effet s'estompe à l'âge adulte. Il n'y a en revanche pas de corrélation statistiquement significative entre les effets additifs des capacités d'apprentissage et des autres traits, ce qui indique encore une fois que la corrélation observée entre les capacités d'apprentissage et la viabilité dans la première partie du projet était due à des effets d'allèles partiellement récessifs.

Au, final, mes résultats montrent bien l'existence d'une variabilité génétique pour les capacités d'apprentissage, et l'expérience du diallèle montre que la variance additive de cette capacité est importante, ce qui permet une réponse à la sélection naturelle. Toutes les lignées, consanguines ou isofemelles, ont obtenu des scores d'apprentissage supérieurs à zéro. Ceci suggère que les allèles supprimant les capacités d'apprentissage sont fortement contre-sélectionnés dans la nature Néanmoins, mes résultats ne peuvent pas expliquer le maintien de cette variabilité génétique par eux-même. Même si l'hypothèse de pléiotropie entre les capacités d'apprentissage et l'un des traits liés à la fitness que j'ai mesuré ne peut être éliminée, il n'y a aucune preuve d'un compromis évolutif pouvant contribuer au maintien de la variabilité.

Part I

Introduction

In the study of animal behavior, learning ability is a particularly central theme because it simultaneously allows behavioral plasticity and adaptation to a changing environment during a lifetime. Centered on the importance of learning ability in animals, the debate «nature vs nurture» has been of crucial importance during the 20th century, drastically opposing learning to instinct to explain behaviors. However, recently the respective roles of learned and innate components of behavior in animals are being more clearly understood in the light of evolutionary biology [127]. Learning ability and memory are widespread in all animal kingdom, at different levels. Nevertheless, its evolutionary aspect are not well-known. Evolution relies on natural variation on which selection and genetic drift can act. Is there still genetic natural variation in learning ability? How is this variability structured? Does it interact with other traits?

Different forms of learning

Learning is defined as the ability of an individual to modify its behavior in response to past experience, and memory as the ability to store the learned information and thus maintain the modified behavior for variable time periods [41]. An alternative definition of learning is "acquisition of neuronal information of new representation" [53]. But if learning ability is widespread amongst animals, invertebrates as well as vertebrates [172, 49], simple forms like habituation have also been characterised in ciliates like *Stentor coeruleus* [197] suggesting that learning can also occur in organisms without neural networks.

Learning is a complex phenomenon that can be expressed in different forms. The simplest forms of learning are habituation, i.e. the decrease of the behavioral response to repeated exposure to a stimulus, and sensitization, i.e. the increase of this response. These two simple forms of learning have been demonstrated in Aplysia [87] by experiments based on the siphon and gill withdrawal reflex. However, both habituation and sensitization are non-associative forms of learning, because the stimulus cannot be predicted on the basis of an environmental cue. Associative learning has first been demonstrated by Pavlov's experiments [146]. In this case, organisms learn to associate two stimuli, a neutral one and an "unconditioned" one. This unconditioned stimulus can be negative (punishment) or positive (reward). During the conditioning cycle, the neutral stimulus becomes "conditioned". After one or several conditioning cycles (paired presentations of the two stimuli), the conditioned stimulus is recognized alone as a signal for reward or punishment, and the animal produces a "conditioned response", for example salivation for a food reward. Classical conditioning is based on reflex responses, like

salivation. Operant conditioning relies on "learning by doing", on motor response and decision making [177, 157]. Operant conditioning has for example been investigated in the fruit fly *Drosophila melanogaster,* using a "flight simulator". In this device, a flies attached in the middle of an arena can choose the direction in which to fly, according to visual patterns displayed in the arena walls, associated to aversive (heat) stimuli [154, 195, 196]. Other complex forms of learning exist, such as social learning, which is based on imitation. Social learning was first demonstrated in invertebrates by Darwin, who observed the foraging behavior of honeybees that copied bumblebees [37]. Ever since social learning has almost exclusively been studied in vertebrates, including humans. Learning ability in insects has only been quite recently explored. Although as suggested by Mayr [119], insect behavior could be mainly dominated by innate preferences and patterns, it has nevertheless been shown that many insect species demonstrate associative learning abilities. Demonstrating associative learning for example in insects like grasshopers [54], honeybees [125], parasitoid wasps [105, 144], butterflies[121] and flies [153], has finally shown only recently, that learning plays a central part in insect behaviors [91] and this includes social learning [36].

Evolution of learning ability

Learning is assumed to give fitness advantages only when individuals are exposed to moderate environmental changes. In a stable environment, innate behaviors are more useful because learning carries costs [84, 183, 50] of two types: first, the time required to learn and the impact of initial mistakes and second, the energy needed, both for neuron maintenance and signaling [103], and for memory formation itself [129, 23, 96]. Since this energy cannot be invested in other life-history functions, it can negatively affect survival and/or reproduction. Alternatively, learning may not be advantageous if the environment changes so quickly that the experiences are unrepeatable, and cues for anticipating environmental resources or threats are unreliable. But in a moderately changing environment, the benefits of learning ability outcome its costs. For example, grasshoppers living in a variable environment had a better growth rate if they were able to learn to recognize the food of good quality [54]. In this study, grasshoppers were placed in artificial environment and provided two qualities of food, one favoring growth rate and another that was poorer in quality. The animals which were allowed to use environmental cues to learn to recognize the good food grew significantly better than the others for which the environment gave no cue to find the good resources (randomly changing association of spatial location, taste and color of the well-balanced food). It has also been shown in butterflies that species living in a relatively constant environment, with only a few specific host plants, show poor memory ability [145, 35]. In 2009, Dunlap and Stephens performed experimental evolution on *Drosophila,* using replicate populations in differently changing environments during 30 generations. They provided another experi-

mental demonstration that learning is favored by some types of environmental change while selection acts against it in other cases [55]. These experimental studies thus provides an evidence for the adaptive significance of learning.

Some aspects of the evolutionary biology of learning remain unclear, especially the amount of natural variability in learning within species [51, 52]. To understand how learning, as a trait, evolved and continues to evolve, it is necessary to study the genetics of its natural variation in populations, and its dynamics. With no genetic variation, evolution cannot happen. Gene variants occur through mutations that are mostly deleterious and hence either eliminated from the population by selection or maintained at a very low frequency [176]. In this case, genetic variation within populations is maintained by mutation/genetic drift/selection equilibrium. Additionally, genetic variation for fitness-related traits can also be maintained by balancing selection. This can be due to five different factors. Genotype-environment interaction: a spatially or temporally heterogeneous environment, in time or space, induces variation in selection [104, 70, 67, 94]; genotype-sex interaction: selection pressure differs between males and females; frequency-dependent selection: selection pressure varies with the frequency of the phenotype; overdominance: heterozygous individuals have a higher fitness than both homozygotes; antagonistic pleiotropy: a trade-off between two traits in which the same genes are involved [160, 165, 60]. It has been argued that antagonistic pleiotropy could act to maintain genetic variation [60, 160] witout the help of other mechanisms. As most newly arisen mutations are deleterious, the most frequent ones in the population are supposed to be highly advantageous for one pleiotropic effect, but deleterious for others [149]. Nevertheless, according to Hedrick [75], for two traits A and B, considering one locus with two alleles (1 and 2) and assuming the components of fitness are multiplicative [164, 75], the conditions for stable polymorphism would be reached under this assumption:

$$A_{11}B_{11} < A_{12}B_{12} > A_{22}B_{22}$$

The geometric mean of the heterozygotes has to be higher than the geometric mean of the homozygotes [70], which is a case of overdominance. On the opposite, Rose [164] argued that antagonistic pleiotropy could maintain polymorphism if it is associated with directional dominance. In this case, for one gene with two alleles, the effect of the first fitness component is higher for one homozygote compared to the two other genotypes. The effect of the other fitness component is higher for the other homozygote compared to the other two other genotypes. Some other mechanisms can help slowing erosion of variation, but not maintain variation in absence of other factors, like correlational selection which favors combinations of traits and works similarly to antagonistic pleiotropy [161].

Most of the observable genetic variation, especially in behaviors, like learning ability, is quantitative [111] which implies that most phenotypic, morphological and behavioural traits

are usually affected by large number of genes [60, 109, 64] with a complex architecture, interacting with a large number of environmental factors. The genetic variance of such traits can be separated into different components: additive variance, dominance variance, epistasis variance. Fisher [63] suggested that natural selection mostly acts on additive genetic variants. However the amount of additive versus non-additive variation expected in complex traits such as behaviors is highly debated. According to some authors [123], non-additive genetic effects represent most of the variation.

Even if genetic variation is known for all the cognitive traits (review, see [51]), only few species have been specifically tested for their natural genetic variation in learning ability. Such variation is known from association studies in human (review, see [51]). Most of the genetic polymorphism observed for learning ability has been shown with artificial selection experiments. In rats, Fuller and Thompson [65] selected good and bad spatial learners and controlled that the observed differences was not due to motivational or emotional variables. Genetic variation in learning ability has also been experimentally demonstrated in several insect species. In honeybees *Apis mellifera*, [19, 20, 26], populations were successfully selected for good and poor learning ability over a few generations in an artificial selection experiment. In *Leptopilina boulardi*, a small parasitoïd wasp, Perez-Maluf *et al* [151] found genetic variation for the odor conditioned probing behavior, which allows the insect to learn to locate its hosts. The study of two generations of isofemale lines indicated that the latency and the probing duration varied under genetic control in a wild population. In blowflies, *Formia regina [120]* and fruit flies *Drosophila melanogaster* [107], widely divergent population have been selected using a protocol relying on association between stimuli and a sugar reward. Also in *Drosophila melanogaster*, an other artificial selection experiment based on aversive conditioning allowed selection for high learning ability in flies derived from population with low learning ability [128]. Moreover, in this species, one locus, named *foraging* is known to be polymorphic in nature. It affects several phenotypic traits, including foraging behavior in larvae [178], recognition of attractive odorants in a foraging context [171], adult locomotory behavior after feeding [148] and also learning ability in adults [126] and larvae [90]. The *foraging* gene encodes a cGMP-dependent protein kinase (PKG) [142]. In mammals, PKG is involved in synaptic plasticity and learning [61, 78, 95]. Two alleles are known, for^R (rover) and for^S (sitter). In flies, rover larvae tend to move more than sitters in presence of food and show a higher PKG activity. They also show a greater memory acquisition and retention for appetitive learning [90]. In adults, polymorphism in PKG affects various associative learning phenotypes: the for^R flies have a better short term, but a reduced long term memory relative to the for^S [126]. The *foraging* gene is the best-known natural variant that affects learning ability.

Nevertheless, the question of the natural genetic variation in learning ability in animals

remains largely unexplored. What is the amount of genetic variation for learning ability? If there is genetic variation in learning ability, what portion is additive? Is there genetic correlation between learning and other traits related to fitness?

I investigated this question in the fruit fly *Drosophila melanogaster*, a small insect which belongs to the *Diptera* order. Insects are rather simple organisms, but they still have complex learning abilities. *Drosophila* is a good model for studying learning ability in large populations over several generations, especially due to its short generation time and high fecundity. This has also been a chosen model for intensive neurological studies in the past few decades, especially the neurology of its learning ability.

Drosophila as a biological model for learning studies

Why *Drosophila*? Originally from Africa, *Drosophila* spread to all continents, except Antarctica, following human populations. It thrives on a very wide range of decomposing fruit and vegetable matter. The larvae and adults live on the same resources, even if they show some differences in yeast preferences (adults are more generalists than larvae) [179]. The larval development time is short, about ten days at 25 °C, but slows down at lower temperature or when raised on nutritiously poor diet. Moreover, *Drosophila* are highly fecund, producing large number of progeny thus facilitating fly husbandry in laboratories and allowing for multiple and repeatable measurements on a single strain. *Drosophila* behaviors are rather complex and well described, especially its learning abilities, which have been demonstrated with simple associative learning tests [153, 92], classical conditioning experiments, and operant conditioning experiments [21]. The first experiment in *Drosophila* based on aversive conditioning was performed by Quinn et al in 1974 [153]. In this experiment, flies learned to avoid odors associated with an aversive electric shock. They were also capable of appetitive conditioning, associating odors to sugar rewards [185]. Moreover, social learning, although still poorly characterized, has been recently described in *Drosophila*. Mery et al [132] have demonstrated that a female *Drosophila* are able to copy the mate choice of other females. Female flies spent more time with males chosen by other females, even if those males are low quality (smaller) compared to other males.

Fruit flies are also able to store information about various features, like visual features [147], food [185], egg-laying sites [128], and conspecifics, like mates [174] or competitors [203]. They can store information in four different memories: short term (STM), middle term (MTM), long term (LTM), and a form of consolidate memory named anesthesia-resistant memory (ARM) [153, 186, 83], which does not involve protein synthesis unlike LTM [189].

Mutagenesis experiments have revealed several neural molecular mechanisms and genes involved in learning and memory processes [189]. Mutants with impaired learning ability, like *dunce*, or r*utabaga*, have been indentified [47, 106]. Most of the genes known to affect mem-

ory and learning ability have been identified by experimental mutagenesis. Consequently, the natural genetic variation in learning abilty is poorly known.

Natural variation in learning: previous studies in *Drosophila* Given the life history of *Drosophila*, learning ability could be advantageous in choosing the best food, both in terms of quality (good nutrients...) and environmental safety (absence of pathogens, predators, disturbance...), because these flies usually live on ephemeral resources, that are heterogeneous in time and space. Time because in temperate climates, fruits are not available all year. Space, because the fruits and vegetables where flies are feeding are displayed in patches which can be separated by large areas (from centimeters to kilometers). Nevertheless, there still are costs corresponding to two different kinds of trade-offs, linked to phenotypical plasticity (physiological) and genetic (evolutionary) [181]. In *Drosophila*, both types of cost have been shown to play a part in determining the extent of learning ability. First, it has been shown that the utilization of memory leads to a greater susceptibility to dehydration and starvation [130]. This plastic cost is ecologically relevant, given the importance of starvation/dessication resistance in *Drosophila* [139]. Nevertheless, these kind of trade-offs only reveals individual phenotypic plasticity instead of genetic variation. A genetic trade-off is illustrated, for example, by the relationship between learning ability and fitnsess-relatied traits. In a study performed by Burger et al [23], flies that have the best learning ability live shorter lives than flies with less learning ability. A similar trade-off has also been demonstrated between larval competitive ability and learning: flies selected for learning show a decrease in larval competitive ability [129]. In contrast, no trade-off has been shown between learning ability and resistance to parasitoïds [97], which confirms what has been found in social insects [6]. Finally, a trade-off also exists between memory phases, depending on a single gene, which has been demonstrated for the *foraging* gene [126]. Such evolutionary trade-offs are common in fitness-related traits, for example in body size: animals that have a large body size may benefit from advantages like higher fertility, or advantages in mating competition [17], but also suffer from costs like increased juvenile developmental period, or a weak resistance to starvation [168]. These genetic trade-offs associated with learning ability may contribute to maintenance of genetic variation, especially in variable environments, because selection may favor different strategies. Nevertheless, we do not know how many genes contribute to this variation, nor their interaction (epistasis, dominance), allelic diversity, exact roles or locations.

Inbred and isofemale lines: tools to study natural polymorphism

To study natural genetic variation of a behavioral trait, it is generally not possible to use direct observation of allelic polymorphism, via molecular biology methods, like electrophore-

sis [140], because neither the number of genes involved in the expression of phenotype nor their location nor the markers linked to the observed trait are known. Then, to partition phenotypic variance into its different components, all methods are based on the same principle: phenotypic resemblance between relatives provides information on the degree of genetic resemblance among individuals [109]. The purpose is first to separate the phenotypic variance observed in the population into two components: environmental variance and genetic variance and then, the genetic variance into additive, dominance and epistasis variance, if possible. Analysis of family trees over several generations, calculation of coefficients of identity of a gene between two individuals are methods commonly used to investigate this degree of resemblance between relatives. Another method is to create artificial lines of related individuals. It is therefore necessary to use a method that allows the alleles presents in the outbred base populations to segregate in different families, or even homozygous lines. There are two complementary approaches: using lines obtained after several generations of inbreeding, which allows study of groups of individuals that share the same genotype, or using newly initiated isofemale lines. Isofemale lines are produced from one couple randomly extracted from natural outbred population. Depending on their number, they are quite representative of natural outbred based population, but as they combine four haplotypes, they display some genetic variance. On the other hand, inbred lines are also randomly extracted from the natural outbred-base population, but their within line variance is close to zero [60]. Nevertheless, they can suffer some inbreeding depression resulting in the lost of the less viable/fertile genotypes. Their behavior may also suffer from this inbreeding depression.

Effects of inbreeding on behavioral traits Inbreeding is the result of mating between individuals related to each other by ancestry [60]. If we derive several inbred lines from an outbred base population, the genetic variance within the lines will decrease, because inbreeding over several generations leads to allele fixation (see fig. 1), whereas the genetic variance between lines will increase [60]. Allele fixation within lines leads to individual homozygosity at all loci. This may lead to inbreeding depression, i.e. a decrease of the mean of a trait over all inbred lines. Two main causes of inbreeding depression have been identified: overdominance [173, 56, 16], which means that heterozygotes have a better mean for the measured trait than both homozygotes and directional dominance, which means that the homozygote for one allele and the heterozygote has a higher mean than the homozygote for the other allele [38, 29]. Epistasy can also play a part as a third cause, althought it is more difficult to measure.

Effects of inbreeding on behavioral traits have been less investigated in comparison to life-history or morphological traits. Direct measures of fitness, like lifetime reproductive success, are a common measured trait, but some studies (see below) suggest that inbreeding also causes decline in behavioral traits that are presumably directly related to fitness.

The effects of inbreeding on competitive ability, which is related to survivorship and fitness, have been measured on male mice [122]. Inbreeding reduces their fitness, as inbred male mice only sired one-fifth of the offspring than outbred males. It is interesting to note that this does not occur under laboratory conditions, but only in semi-natural enclosures, which shows the role of environmental conditions and variation of selection pressures between laboratory and more natural conditions. In Salmon fish, less inbred individuals are more aggressive and have a higher specific growth rate than mildly inbred fish, leading to their higher survival in high-density competitive environment, even though they show equal ability to establish territorial dominance in low density environment [66].

The effects of inbreeding on sexually selected traits have also been investigated, as they are strongly related to fitness. Concerning vertebrates, courtship behavior in guppies [187] is significantly reduced with inbreeding. Mariette et al [118] also showed that male sexual motivation of guppy fish *Poecilia reticulata,* defined by courtship intensity and following behavior, and mating success are affected by a single generation of full-sib mating. Only one generation of inbreeding (full-sib mating) significantly reduces male reproductive performance in *Heterandria formosa*, a poeciliid fish [4]. In black grouse (*Tetrao tetrix*) the lifetime copulation success, and the ability to obtain a central lek, is greater for males that display high heterozygosity level (measured on 15 microsatellite loci) [81]. Margulis and Atman [117] observed that inbreeding affected reproductive success of females from one subspecies of old-field mice *Peromyscus polionotus* (suggesting it may affect behavior), but not in another subspecies. In the house mice, copulatory behavior is also negatively affected by inbreeding (shorter latencies to the first mount and intromission, longer latencies to ejaculation, and more pre-ejaculatory mounts and thrusts [45]. Also in the house mice, male aggressive behavior and competitive ability decreases with strong inbreeding [59]. In male song sparrow, the song repertoire is reduced with increasing inbreeding [155].

Male mating success is also affected by inbreeding in insects. In houseflies, bottleneck episodes have been shown to lead to divergence of courtship behavior [124]. Sharp [170] demonstrated that male mating ability in *D. melanogaster* was also significantly decreased by inbreeding. The decline was linear during 18 generations of inbreeding (between 5.9 and 10.7% decrease per 10% increase in F). A decade later, Miller *et al* [135] observed that, in *D. melanogaster*, isogenic males for chromosome 2 displayed impaired mating behaviors, and, for 2 of 5 lines, aberrant courtship patterns. In *Drosophila montana*, for male song frequency the average inbreeding depression was 14% [8]. Male song is an important part of courtship, associated with courtship success and offspring survival. The mating success of male butterflies is also decreased by inbreeding, and this result is magnified when the animals are bred in more natural conditions (unconstrained flight) [86].

Other traits have been measured, less directly related to fitness, like human aversion in

pointer dogs. The comparison between inbred and non-inbred strains did not show significant behavioral differences [22]. Concerning the effects of inbreeding on cognitive ability, inbred strains of rats showed a significantly lower level of spatial learning [73] than outbred; however, the control outbred strains were derived from a different genetic background. The effects of inbreeding on learning ability are mainly unknown.

The effects of inbreeding on behavioral traits are mostly deleterious, as observed with other fitness-related traits. Consequently, behaviours that favour mating between non-relative are often considered as inbreeding-avoidance behaviour, althought this hypothesis is discussed [136]. This deleterious effect can be due to recessive deleterious alleles that are expressed in the inbred lines that are usually present in heterozygous state in outbred base populations and therefore masked by dominance. Another explanation is that heterozygous individuals have better performance than either homozygote due to overdominance. If inbreeding has no deleterious effects on a trait, this could reveal a lack of variation, due to very strong selection pressure on the trait or elimination of the genetic variation during a bottleneck process, especially if the animals have been domesticated or kept in the laboratory for a long time as a small population. This could also be due to a purging of the most deleterious alleles during the inbreeding process [31]. In some cases, finally, inbreeding can increase a trait. This can also be due to selection during the inbreeding process. In the red flour beetle, *Tribolium castaneum*, inbreeding has been shown to increase female promiscuity, in order to increase fitness gain for this behaviour in case of inbreeding. Polyandry in this case can possibly reduce the risk of fitness decrease because of mating with males producing sperm carrying genetically incompatible haplotypes [133].

Use of inbred lines to study natural variation Producing inbred lines from a natural population is a powerful tool to study natural variation. Inbred lines represent a sample of the alleles present in nature and are used to precisely identify polymorphism. Transcription analysis can be performed in parallel to identify candidate genes for the measured traits.

For example, in plants, inbred lines as sample of natural populations have been used to quantify variation in herbivore-induced volatile emission in maize and to analyze the nature of this variation (nature of chemical compounds produced by the plant; [42]). In *Drosophila*, several studies have been performed in different species. In *Drosophila* of the virilis group, [13] studied multiple inbred lines of laboratory populations from different species to measure variance in alleles for the effects of heat-denaturation on xanthine deshydrogenase. In *Drosophila melanogaster*, Trudy Mackay's group produced a very useful tool for the study of natural variation, the *Drosophila* Genetic Reference Panel (DGRP): a few hundred lines derived from a natural population of Raleigh (California), inbred for 20 generations of full-sib mating and fully sequenced [112]. A lot of work is currently being done on these lines.

[85] quantified the locomotor behavior variation among DGRP lines and lines selected for different levels of locomotion, in order to quantify variation and identify candidate genes via transcription analysis. [9] measured phenotypic diversity in several fitness-related traits as starvation stress resistance, chill coma recovery, longevity, locomotor reactivity, copulation latency, reproductive fitness and competitive index among 40 DGRP lines. They also measured genome-wide transcript abundance (10096 genetically variable transcripts) in order to identify candidate genes responsible for variation in these traits.

Concerning behavioral traits, Wang et al [192], measured the polymorphism of 13 odor-binding protein genes and the associated odor-recognition behavior among some DGRP lines. [162] measured the ability of flies to respond to benzaldehyde odorants, and the polymorphism of 6 odorant receptor genes. [7] also measured the polymorphism of 6 odorant binding protein genes among some of the DGRP lines , and correlated it with odor response and longevity. Other behavioral traits have been investigated, like aggressive behavior. This behavior has been compared among 40 of the DGRP lines, and candidate genes have been identified [57, 58]. Variation in sleep has also been studied, and quantitative trait transcripts identified (variation in transcripts abundance; [71, 72]).

Nevertheless, inbred lines are not the only tool available to study natural variation. Analysis of recombinant lines and diallel crosses between inbred lines provide more insight into genetic details such as gene location, or the relative role of dominance and additive variance, than the analysis of inbred lines alone.

Isofemale lines Keeping populations for several generations can lead to adaptation to laboratory conditions that differ markedly from the natural environment. Moreover, during the inbreeding process some deleterious alleles that are present in the wild population at a very low frequency, mostly in heterozygotes, will be purged. Finally, genetic drift during inbreeding process in the laboratory will result in further modification of allele frequencies. Inbred lines consequently may have lost a part of the variation of the outbred natural population. To avoid this problem and study lines which still contain a large part of the natural variation of the wild population, one solution is to utilize lines that have been founded from one wild couple (four independent haploid genomes; [40]), during the first generation in the lab. Because of the remaining variability, low frequency deleterious alleles may stay at a heterozygous state. Moreover, as the selection does not have the time to act during several generations, the loss of alleles via hitchicking mechanisms is limited. The loss of isofemale lines is therefore experimentally less important than the loss of inbred lines during the inbreeding process. Nevertheless, the tested lines have necessarily been raised in the lab, because this is a standard and homogeneous environment which will minimize the environmental variance. This is not useful for discrimination between components of genetic variation (diallel crosses of

inbred lines are needed for that), but the observed variation in this scenario will be closer to what exists in the wild.

Overview of the thesis

To investigate the genetics of natural variability of learning ability in *D. melanogaster*, we collected a large sample of a wild population in Valais (Switzerland). First, I inbred several lines in order to investigate the effects of inbreeding on learning ability, study variation in learning between inbred lines, and look at correlations between learning ability and two other fitness related traits. Then, I performed a complete diallel cross of the inbred lines to identify the nature of the genetic variation observed in the inbred lines and to test genetic correlations between learning and other fitness-related traits. As a complementary approach, I sampled again several isofemale lines from the original Valais population in order to measure the same traits as on inbred lines, but on lines that have not adapted to the lab conditions.

Part I In this chapter, I addressed the effect of inbreeding on learning ability. I inbred several lines isolated from a wild population, in order to reveal the effects of recessive alleles segregating in the population. Inbreeding increases the homozygosity level [200]. After twelve generations of sib-mating I obtained several lines which randomly fixed different alleles depending on the initial genetic variation. The effects of inbreeding on learning in *Drosophila* are unknown, but inbreeding is known to alter fitness-related traits [202]. Hence, I investigated if inbreeding depression also affects learning ability. Is there variation between the inbred lines for learning ability and correlations between learning ability and other fitness-related trait?

Part II The first chapter's study revealed genetic variation for learning ability and a positive correlation between the learning ability and the egg-to-adult viability of the natural population. This suggested an effect of pleiotropic recessive alleles. Nevertheless, the effect of such alleles, maybe in small number and great effect, could hide other genetic relationships between the traits. Consequently, I sampled again the natural population from which the inbred lines described in chapter one, in order to derive isofemale lines. I measured learning ability and again three fitness-related traits that may be related to learning ability: resistance to bacterial infection and egg-to-adult viability, in isofemale lines right after their sampling in the wild. This allowed us to avoid any adaptation to lab conditions, and answer the following questions: what is the genetic variance of learning ability between lines that have not been adapted in laboratory conditions and harbour a slight within-line variation allowing to hide the effects of rare highly deleterious alleles? Is there a correlation between their learning ability and other fitness-related traits?

Part III We performed a diallel cross [69, 30] between all inbred lines described in the first chapter, in order to investigate the nature of the genetic variation observed in the first chapter, and the genetics of a positive trade-off observed between learning ability and egg-to-adult viability. Each line was crossed with all the others and tested for learning ability and three main traits possibly related to it: resistance to bacterial infection, egg-to-adult viability and developmental time, in order to answer the following questions: how large are the additive and maternal/paternal contributions in the observed variation? What are the genetic correlations between learning and other fitness-related traits? What is the relationship between the phenotype of inbred lines and their breeding values for these traits? Are the traits we measured affected by inbreeding?

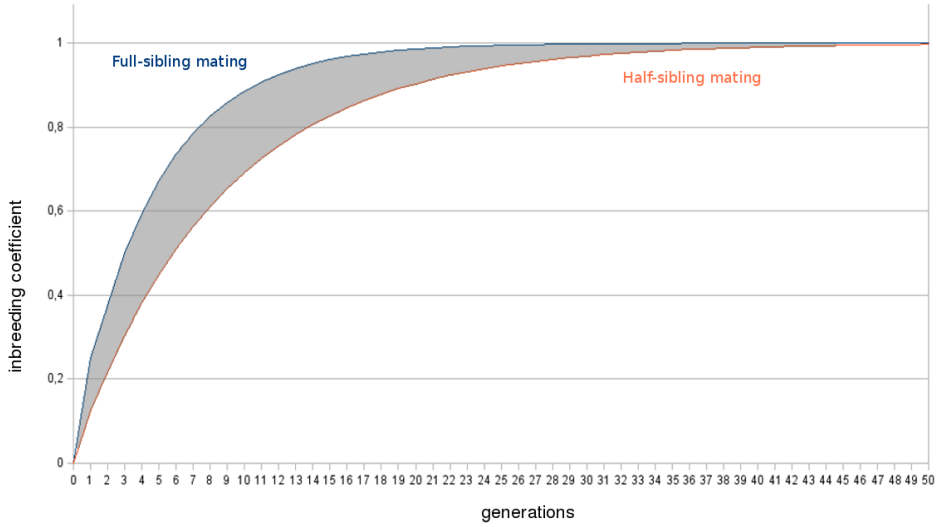

Figure 1: Inbreeding coefficient increased over generations, in case of full-sib and half-sib mating. Our inbred lines have been inbred for twelve generations. As we do not know whether the parents were half or full siblings, because multiple matings occur in *Drosophila*, the inbreeding coefficient of our lines is between the values of full and half siblings.

Part II

Effects of inbreeding on aversive learning in *Drosophila*

Népoux V., Haag C. and Kawecki T. J.

This work has been published in Journal of Evolutionary Biology, vol 23 (2010).

Summary

Inbreeding adversely affects life history traits as well as various other fitness-related traits, but its effect on cognitive traits remains largely unexplored, despite their importance to fitness of many animals under natural conditions. We studied the effects of inbreeding on aversive learning (avoidance of an odor previously associated with mechanical shock) in multiple inbred lines of Drosophila melanogaster derived from a natural population through up to 12 generations of sib mating. While the strongly inbred lines after 12 generations of inbreeding ($0.75 < F < 0.93$) consistently showed reduced egg-to-adult viability (on average by 28 %), the reduction of learning performance varied among assays (average = 18% reduction), being most pronounced for intermediate conditioning intensity. Furthermore, moderately inbred lines ($F = 0.38$) showed no detectable decline in learning performance, but still had reduced egg-to-adult viability, which indicates that overall inbreeding effects on learning are mild. Learning performance varied among strongly inbred lines, indicating the presence of segregating variance for learning in the base population. However, the learning performance of some inbred lines matched that of outbred flies, supporting the dominance rather than the overdominance model of inbreeding depression for this trait. Across the inbred lines, learning performance was positively correlated with the egg-to-adult viability. This positive genetic correlation contradicts a trade-off observed in previous selection experiments and suggests that much of the genetic variation for learning is due to pleiotropic effects of genes affecting functions related to survival. These results suggest that genetic variation that affects learning specifically (rather than pleiotropically through general physiological condition) is either low or mostly due to alleles with additive (semi-dominant) effects.

1 Introduction

Inbreeding arises through mating between relatives and results in increased homozygosity [200, 33, 167]. Inbreeding typically leads to a decline in fitness-related traits, such as survival,

competitive ability, viability, fertility, pathogen resistance etc. [202, 102, 101, 32, 10, 93, 2, 110] a phenomenon known as inbreeding depression [28, 60]. Avoidance of mating with kin, observed in many species [150, 194], suggests that inbreeding depression under natural conditions is strong enough to cause selection for mechanisms that prevent inbreeding.

Two major hypotheses could explain inbreeding depression [28]: overdominance [169], which gives a fitness advantage to heterozygous individuals, and (directional) dominance [38, 202], whereby the increase of homozygosity reveals the effects of recessive deleterious alleles. These two mechanisms are not mutually exclusive, different patterns exist in different species. However, the dominance hypothesis is better supported empirically, at least in *Drosophila*, mice and humans [43].

In contrast to life history, the effects of inbreeding on behavioral and in particular cognitive traits remain poorly known. Among the few existing studies, inbreeding is suggested to cause deficits in parental behavior [117, 116] and copulatory behavior [45] in mice. It is also suggested to affect male courtship behavior in the housefly [124], decrease male mating behavior in fish and butterflies [86], and reduce song repertoire in male song sparrow [155]. The effects of inbreeding on learning ability have been examined in rats, where inbred strains showed a significantly lower result in spatial learning [73]; however, the control outbred strains for that study were derived from a different genetic background. In human populations, correlative studies have found inbreeding to be deleterious to some cognitive functions, like reading or learning ability [12, 3, 166, 1], but these finding are not universal [137]. Moreover, interpretation of these correlative studies can be confounded by other factors, including socio-cultural differences. Marriage between relatives is likely to depend on socio-economic background, which may also affect the results of cognitive performance tests.

Here we study the effects of experimental inbreeding on a cognitive trait - associative learning ability - in *Drosophila melanogaster*. *Drosophila* are capable of learning in response to classical associative conditioning, as well as in operant conditioning (involving motor responses and decision making; [153, 92]). Four memory types have been identified: short term (STM), middle term (MTM), long term (LTM), and a form of consolidated memory named anesthesia-resistant memory (ARM) [152, 186, 83], which does not involve protein synthesis [190]. It has been shown that flies are also able to store information about various features, like visual cues [147], food [185], egg-lying sites [128], and conspecifics, like mates [174] or competitors [203]. As in most species, inbreeding in flies results in deterioration in fitness-related traits, such as competitive ability, viability, fecundity, and male mating success [25, 113, 135, 102, 80, 101]. Among behavioral traits, inbreeding depression affects male song frequency in *Drosophila montana* [8] and reduces male mating ability in D. *melanogaster* [170, 135]. Moreover, artificial selection for improved learning ability performed on small populations actually led to a decline in learning performance, presumably due to inbreeding

depression [76].

Positive responses to experimental selection on learning performance in other experiments [107, 128, 131, 55] show that *Drosophila* populations harbor natural genetic variation in learning ability; a specific natural polymorphism contributing to this variation has been identified [126, 89]. Correlated responses to selection revealed negative additive genetic correlations of learning performance with larval competitive ability and adult lifespan, presumably reflecting evolutionary trade-offs [129, 23, 96]. In order to gain insights into the genetic architecture of learning ability, we used multiple inbred lines of *Drosophila* derived by sib-mating from a base population recently acquired from the field. We ask the following questions.

First, does learning performance show inbreeding depression, and how strong is it, compared to inbreeding depression for egg-to-adult viability, for which inbreeding depression is firmly established [113]? Inbreeding depression would indicate that polymorphisms affecting learning performance segregate in the base population, and that the alleles that reduce learning are, on average, recessive, partially recessive, or overdominant.

Second, is there variation among the inbred lines, and do all of them show inferior learning performance and viability compared to the outbred base population? Because different inbred lines become randomly fixed for different alleles, variation among inbred lines captures a part of the genetic variation present in the base population. Variation among inbred lines would help to interpret potential absence of inbreeding depression as being due to additivity of allelic effects (i.e., semi-dominance) rather than due to lack of genetic variation in the base population. Furthermore, if there were on average some inbreeding depression but some of the inbred lines were equal or superior to the outbred population, it would indicate that heterozygosity is not required for high learning ability, which would support the dominance rather than the overdominance hypothesis main mechanism of inbreeding depression.

Third, does learning performance of individual inbred lines correlate with their egg-to-adult viability? Such correlation would suggest pleiotropy. A positive correlation would suggest that inbreeding depression is mostly due to alleles that impair some general functions of the organism affecting both life history and learning performance. On the contrary, a negative correlation between the fitness components and learning performance would suggest a trade-off, similar to trade-offs between learning and competitiveness [130] or lifespan [23] revealed by selection experiments.

Fourth, is there evidence for purging of alleles that reduce learning? Purging of recessive alleles that impair learning might occur if they also impair fitness under the experimental conditions, leading to selective loss of some lines. Under purging, estimates of inbreeding depression from early generations (before line loss) are expected to be larger than estimates from surviving lines later in the experiment. Purging should result in F1 crosses between inbred lines showing on average superior learning performance compared to the base population[31].

2 Material and Methods

We first describe how the inbred lines and the outbred controls were derived. In the subsequent sections we describe the phenotypes were assayed, and how they were used to assess inbreeding depression, performance of crosses, and variation among inbred lines.

2.1 Base population and inbred lines

The base population originated from 400 flies collected in Valais (Switzerland), in October 2007. It was maintained in a large population cage at the size of about 1200 adults and a generation time of three weeks on a yeast cornmeal medium [39], at 25 °C, 60% humidity, and 12:12 h light:dark cycle. The inbred flies were raised the same way except for the density of population.

Inbred lines were produced by sib-mating. A mated female was isolated and allowed to oviposit. Her offspring were then allowed to mate among themselves upon emergence, and a new mated female was isolated and used to establish the next generation. Multiple mating is common in *Drosophila* [134, 82], and thus, the offspring of a randomly chosen mated female may have several fathers, allowing for the possibility of half-sib rather than full-sib mating in our experiment. The coefficients of inbreeding F was thus bound by the following the recurrence equations [141]:

$F_{t+1} = 1/4(1 + F_{t-1} + 2F_t)$ (assuming full-sib mating, maximum inbreeding)

$F_{t+1} = 1/8(1 + 6F_t + F_{t-1})$ (assuming half-sib mating, minimum inbreeding)

To compensate for the anticipated loss of lines due to fixation of highly deleterious alleles we initially established 50 parallel lines. After 12 generations of sib-mating, the surviving 15 inbred lines were expanded to around 50-100 individuals and subsequently maintained at this size to reduce losses due to demographic stochasticity. By that time, the expected inbreeding coefficient was between 0.75 (assuming all matings were between half-sib) and 0.93 (assuming all mating begin full-sib); with 50 % of each type of mating F would be 0.88.

Many of the original inbred lines were lost in the course of inbreeding, and this process was unlikely to be random with respect to viability effects of alleles being fixed, leading to some purging of such deleterious alleles [31]. Through pleiotropic effects of genes affecting line loss, such purging might have also affected the observed inbreeding depression for learning performance. Therefore, at a later stage, we independently derived additional 15 inbred lines from the same base population. These "moderately inbred lines" were obtained by two generation of full-sib mating (F=0.38) under the same environmental conditions as described above. Full-sib mating was ensured by isolating virgin females and subsequent controlled mating with a single randomly selected male. None of these additional lines were lost, so

they are more representative of the base gene pool. Their viability and learning performance were compared to the original highly inbred lines in a simultaneous (cross-sectional) assay.

2.2 Phenotypic assays

Learning performance. Flies for the learning assays were raised from eggs laid in mass oviposition during two days in 200 ml vial containing 30 ml of standard food. When needed (inbreeding depression and crosses experiments, see below), the emerging adults were anesthetized with CO_2 and mixed, then separated in groups of 60 flies, in 60 ml vials containing 10 ml of food. If CO_2 was used, the flies had at least 24 h to recover before being assayed.

The learning assay involved an association between an odor (conditioned stimulus) and an aversive mechanical shock (unconditioned stimulus; [92]. Flies were conditioned and tested in groups of around 60 individuals (sexes mixed), aged 7 to 10 days. Conditioning consisted of one or several conditioning cycles. In each conditioning cycle the group of flies was first exposed for 30 seconds to one odor (the conditioned stimulus) and simultaneously subject to mechanical shock delivered by a test tube shaker (1 s of shocks every 5 seconds), followed by 60 seconds humid air flow, 30 seconds of the second odor (the neutral stimulus); another 60 seconds period of humid air flow completed the conditioning cycle. When several conditioning cycles were used (to increase the total exposure to of conditioning), they immediately followed one another. Octanol and 4-methyl-cyclohexanol (MCH) dissolved in paraffin (0.6 ml per litter of paraffin) were alternately used as conditioning and neutral stimulus. Both odors are innately avoided by the flies.

A set time after the end of conditioning the flies were placed in a T-maze and allowed to choose between the odors for 45 s. To obtain an estimate of preference, the flies in each arm of the T-maze were counted; flies remaining in the central chamber of the T-maze were ignored. The assays were paired; each group of flies conditioned to avoid octanol was paired with a group conditioned to avoid MCH. One learning score was calculated for each such pair, as the difference in the proportion of flies choosing octanol between the group conditioned to avoid MCH and the group conditioned to avoid octanol. Learning scores were then used as dependent variable in ANOVA after checking for homogeneity of variance (Bartlett test) and normality of residues (visually controlled with Q-Q plot).

Unconditioned responses to odors. The response to odors (odor avoidance) without prior conditioning (i.e., in naïve flies) was also measured. The flies were subjected to the same pattern of shock as in the conditioning procedure, but without exposure to odors. They were then transferred to the T-maze and allowed to choose between one odor (octanol or MCH) and the solvent (paraffin oil). The proportion of flies choosing the solvent indicates their innate tendency to avoid the odor.

Egg-to-adult viability. Eggs were collected in mass oviposition on fruit jelly overnight. One-hundred eggs were transferred to a 60 ml vial containing 10 ml of food; eggs that were infertile (transparent) or mechanically damaged were eliminated. In some cases some lines did not lay enough eggs, in which case some vials were set up with fewer than 100 eggs (see below). To assess viability, we counted the number of adults that emerged within 9 days (normal food) or within12 days (poor food), counting from emergence of the first fly. The proportion of eggs that resulted in an emerged adult was used as an estimate of viability (one value per vial).

2.2.1 *Inbreeding depression*

General design. The inbred lines were assessed for inbreeding depression after five generations of inbreeding (viability), after eight generations (viability and preliminary assessment of learning performance), and after twelve generations of inbreeding (viability, detailed assessment of learning performance, and unconditioned odor responses). Viability tests and a restricted set of learning performance tests were also carried out for the independently derived "moderately inbred lines" (see above). Theses lines were assessed in a "cross-sectional" experiment in parallel with the "strongly inbred lines" (12 generations of inbreeding) and with the outbred controls.

Learning performance. Inbreeding depression is quantified as the proportional reduction of mean performance of inbred individuals. Learning assays were done in groups of 60 adults (see above). Rather than forming each group using a single inbred line, we mixed equal numbers of adults from each inbred line, and the groups of 60 flies were derived from this mixed population. This was done to reduce the variance among the replicates and thus to increase the precision of the mean estimate while not exceeding the number of replicates that could technically be handled. This allowed us to study the average effect of inbreeding on learning performance under a varying number of conditioning cycles (memory acquisition) and a range of time between conditioning and test (memory decay). In all assays described below the outbred flies from the base population served as controls.

The first assay was performed after 8 generation of inbreeding; flies originating from 24 inbred lines we assayed for 20 min memory after two conditioning cycles. After the inbred lines completed 12 generations of brother-sister mating we performed more extensive assays. They included:

(A) The acquisition of short-term memory: the learning scores were assayed about 4 (range 2 to 6) minutes after a varying number (1-5) of conditioning cycles.

(B) The acquisition of middle term memory: the learning scores assayed 60 min after 1 to 3 conditioning cycles.

(C) The memory decay: the learning scores assayed after 5 conditioning cycles as a function

of the interval between conditioning and test (5 min, 1 h, 4 h, 19 h).
Assay (B) was done immediately after the 12 generations of inbreeding were completed and included flies from 20 inbred lines surviving at this point. Five of these lines were subsequently lost and assays (A) and (C) were done on flies originating from the remaining 15 inbred lines.
Finally, we compared the learning performance of flies from 15 highly inbred flies ($0.75<F<0.93$), 15 moderately inbred lines ($F=0.38$), and outbred flies in a single "cross-sectional" experiment. We assessed their short term memory after 3 conditioning cycles, which was the measure of learning performance that showed most pronounced inbreeding depression in the other experiments.

The learning scores were subject to an ANOVA, with inbreeding status and, where applicable, number of conditioning cycles or time between conditioning and testing treated as categorical fixed factors. Where applicable, the initial model also included the interaction between the fixed factors; if not significant, this interaction was removed from the final model reported in the Results. Some of the experiments were performed over two or more experimental sessions, treated as random blocks. We only mention block effects when they were significant; the same applies to interactions between block and other factors. Non-significant block interactions were taken out from the model.

Unconditioned responses to odors. To see whether the effects of inbreeding on learning could have been confounded by differences in unconditioned odor responses, we also studied the effect of inbreeding on the responses to odors (odor avoidance) of naïve flies as described above. This was done after 12 generations of inbreeding on flies originating from 14 inbreed lines, mixed as for the learning assay, as well as on outbred flies. The proportion of flies choosing the solvent was treated as a dependent variable in an ANOVA, with inbreeding status and odorant as fixed factors and block (experimental session) as a random factor.

Egg-to-adult viability. To estimate the inbreeding depression on egg-to-adult viability, three different experiments were conducted. Experiment 1 compared inbred flies from 40 lines after five generations of sib-mating ($0.5<F<0.67$, 40 lines), to the outbred base population ($N=10$ vials). In experiment 2, flies from 20 lines remaining after eight generations of sib-mating ($0.61<F<0.83$) were compared with the outbred base population ($N=5$ vials). In these two experiments, each vial in the inbred treatment was set up with a mix of eggs from four lines, each contributing 25 eggs to the total of 100. Different sets of four lines were used to set up each vial, and all lines were equally represented in the experiments. The data of these two experiments were analyzed with a Mann-Whitney test comparing inbred and outbred flies. In experiment 3, the viability of highly inbred lines (12 generations of sib mating, $0.75<F<0.93$) were compared to outbred flies as well as to moderately inbred flies ($F=0.38$). In this experiment each vial was set up with 100 eggs from a single line, with 2-4 vials for each of 12 highly inbred and 14 moderately inbred lines and 30 vials with the

outbred flies. The data from experiment 3 were analyzed with a generalized linear model with quasi-binomial error to correct for overdispersion.

2.2.2 *Crosses between inbred lines*

To assess whether purging of alleles that impair learning performance or viability had occurred during the inbreeding process, we assessed the average learning performance and egg-to-adult viability of three types of flies: our highly inbred lines, F1 crosses between flies from different highly inbred lines, and the outbred flies from the base population. The parents of all the animals used in these experiments were raised under standard conditions. To obtain the crosses,

14 highly inbred lines (12 generations of sib mating) were crossed in a circular scheme, with line 1 crossed with line 2, line 2 with line 3, ..., line 14 with line 1; each line thus provided the dams for one cross and sires for another. For each cross, eggs were collected from five females and five males; this corresponded to the number of virgin females available from the least productive inbred line. The inbred and outbred flies were raised the same way. The individuals tested for egg-to-adult viability and learning were produced from the same parents.

For learning performance, equal numbers of flies from the 14 inbred lines were combined to create a mixed inbred population; adults from the 14 crosses were likewise combined to obtain a mixed F1 population. These two populations and the outbred population were then assayed for short term memory after 3 cycles of conditioning, as well as for unconditioned responses to odors. The learning scores were analyzed with an ANOVA, with inbreeding status (outbred, inbred and crossed) as the fixed factor and block (defined by three experimental sessions) as a random factor. The odor avoidance scores were likewise analyzed with an ANOVA, treating inbreeding status and odorant as fixed effects and experimental session as a random block effect.

We also measured the egg-to-adult viability of the three categories of flies (inbred, crosses and outbred) on normal food, as well as on poor food containing 10% of yeast used in normal food. Within each category, the eggs were randomly distributed among vials, each vial set up with eggs from up to 4 lines, according to egg availability. Three of the 14 inbred lines did not produce enough eggs for this assay, and some other lines had poor fertility, so the target 100 eggs per vial not always could be reached. Specifically, on normal food, 32% of the vials contained between 75 and 100 eggs, and 11% fewer than 75. On poor food, 17% of the vials contained between 75 and 100 eggs, and 8% fewer than 75. For each vial, viability was calculated as the number of adult flies emerged per vial divided by the number of eggs originally placed in this vial. These values were subject to an ANOVA, with inbreeding status and food type and their interaction as fixed effects.

2.2.3 *Variation among inbred lines*

Learning performance. Because of the labor intensity of the learning assays, for the variation among inbred lines we concentrated on the learning assay for which the average effect of inbreeding was most pronounced, that is, on short-term memory after 3 cycles of conditioning. This was done on 14 highly inbred lines (12 generations of inbreeding $0.75 < F < 0.93$), with 9-14 replicate learning scores per line.

Egg-to-adult viability. Fourteen inbred lines were included, with 4-7 replicate vials per line with 100 eggs each. Some lines had poor fertility, so ten vials (out of 97) contained fewer than the target 100 eggs (15-90 eggs; one vial in line 35, two in line 13, five in line 14 and two in line 48). Seven replicates from the outbred base population were also included. The viability of the flies was assessed as described above.

The learning scores and viability values were checked for homogeneity of variance (with Bartlett's test), and normality of residuals (visually controlled with normal probability Q-Q plot). One-way ANOVA with inbred line as the (random) factor was used to estimate the among-line variance component and test for its significance. Additionally, each line was compared to the outbred population with Dunnett's test. For each line we also used a t-test with the null hypothesis that its mean learning score is zero. Finally, the normality of the distribution of line means was tested with Anderson-Darling normality test.

3 Results

3.1 Inbreeding depression

Learning performance. After eight generations of brother-sister mating the inbred flies tended to show only slightly poorer short-term memory (learning score 20 min after two conditioning cycles 0.59 ± 0.04) than the outbred controls (0.64 ± 0.03; mean $\pm$SE; $F_{1,20}=1.2$, $P=0.28$, $N=11$).

More extensive assays carried after 12 generations of brother-sister mating provided more convincing evidence of inbreeding depression affecting learning. Specifically, for short-term memory acquisition (Fig. 2A), inbred flies showed significantly lower learning scores than outbred flies (ANOVA, $F_{1,82}=13$, $P=0.0005$). The effect was more pronounced for intermediate conditioning intensity (2-4 conditioning cycles), although the interaction between inbreeding status and cycle number was not significant ($F_{4,78}=0.64$, $P=0.63$; the interaction was eventually removed from the model). A similar result was observed for middle-term memory (Fig. 2B), where the inbred flies also performed less well than outbred ($F_{1,75}=5.46$, $P=0.02$), with the effect most pronounced after 2 conditioning cycles, even though the interaction between inbreeding status and cycle number was again not significant ($F_{2,73}=0.74$, $P=0.48$). In a

separate memory decay experiment (Fig. 2C) we detected no effect of inbreeding on memory after five conditioning cycles and the way it declined with time between conditioning and testing (inbreeding status: $F_{1,55}$=0.23, P=0.63; inbreeding × time interaction: $F_{3,52}$=0.18, P=0.91 , removed from the final model). There was no block effect ($F_{1,55}$=0.48, P=0.5) but the block × time interaction was significant ($F_{3,55}$=3.59, P=0.02). As expected, the learning scores declined after 1 h (time between conditioning and test: $F_{3,55}$=94.40, $P<$ 0.0001), although more abruptly than expected, so that the learning scores after 4 h and 19 h were not distinguishable from zero. The short-term memory learning scores after five conditioning cycles in the experiments presented in figure 2A and 2C did not differ significantly between experiments ($F_{1,33}$=2.43, P=0.13).

Finally, we did a cross sectional study including, in addition to the outbred and the highly inbred flies also moderately inbred flies from a new set of lines subject to two generations of full-sib mating (F=0.38). We assayed these flies for short-term memory after three conditioning cycles, under the conditions that previously allowed us to detect inbreeding depression for learning (Fig. 2A). Yet, in this experiment both highly (mean learning score ± SE: 0.55± 0.03) and moderately inbred (0.52 ± 0.03) flies only tended to be slightly inferior to the outbred flies (0.61 ± 0,03 ; $F_{2,45}$=2.18, P=0.12, N=16). Averaged over all assays on lines subject to 12 generations of sib mating, the inbreeding depression for learning performance (the proportional reduction of the learning score) was about 18 %.

Unconditioned responses to odors. Inbreeding did not affect the response to odors (Anova, $F_{1,28}$=0.11, P=0.74; block effect: $F_{1,28}$=18.83, P=0.0002). Both odors were avoided, octanol slightly more (Anova, $F_{1,28}$=20.26, P=0.0001). These results indicate that inbred and outbred flies had the same olfactory response in the absence of conditioning, and thus the inbreeding effects on learning performance reported above were not due to decreased odor detection abilities of the inbred flies.

Egg-to-adult viability. In contrast to learning, the evidence for inbreeding depression for viability was unambiguous in all three experiments (fig. 4; experiment 1: W=0, P=0.01; experiment 2: W=0, P=0.0002; experiment 3, GLM: χ^2=89.9, *d.f.*=2, $P<$ 0.0001). Averaged over the three experiments, 12 generations of sib mating led to 28 % reduction in viability.

3.2 Crosses between inbred lines

Analysis of crosses between inbred lines revealed no evidence that deleterious alleles had been purged during the course of inbreeding. In contrast to the prediction of the purging hypothesis, the viability of the crosses was intermediate between inbred and outbred flies (Fig. 5A; ANOVA $F_{2,86}$=31.5, $P < 0.0001$, Tukey test $P < 0.05$). Even though, as expected, viability was lower on poor food ($F_{1,86}$=25.1, $P < 0.0001$), differences among the three treatments were similar (interaction $F_{2,84}$=0.079, P=0.92, removed from the model).

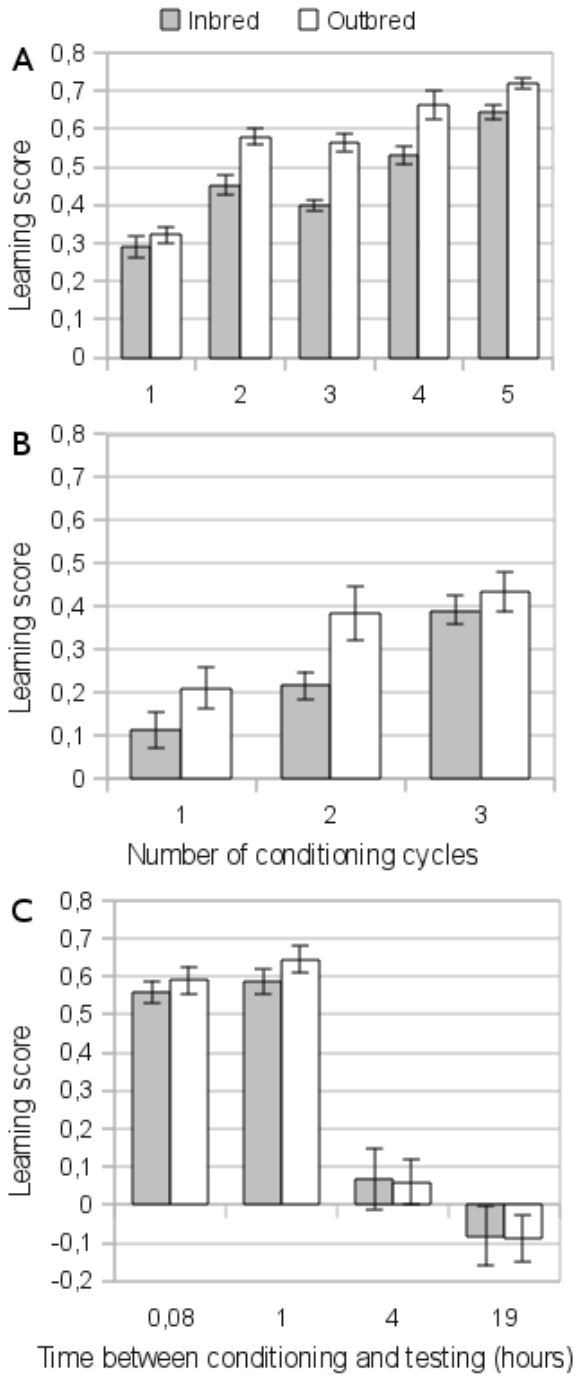

Figure 2: Effects of 12 generations of sib mating on learning performance. (A) Acquisition of short-term memory as a function of the number of conditioning cycles (N=8-10 learning scores per bar). (B) Acquisition of middle-term memory (N=16 per bar for 1 and 3 cycles, and 8 for 2 cycles). (C) Memory decay: learning score after 5 conditioning cycles as a function of time between conditioning and test (N=8 per bar).

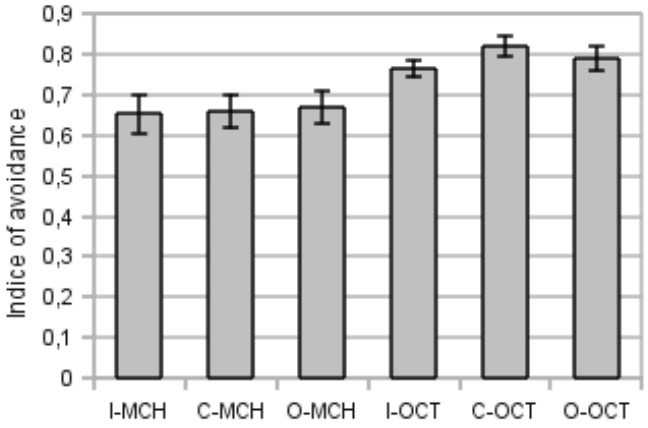

Figure 3: Unconditioned response to odors: the proportion of flies choosing solvent over the odorant (octanol or methyl-cyclo-hexanol).

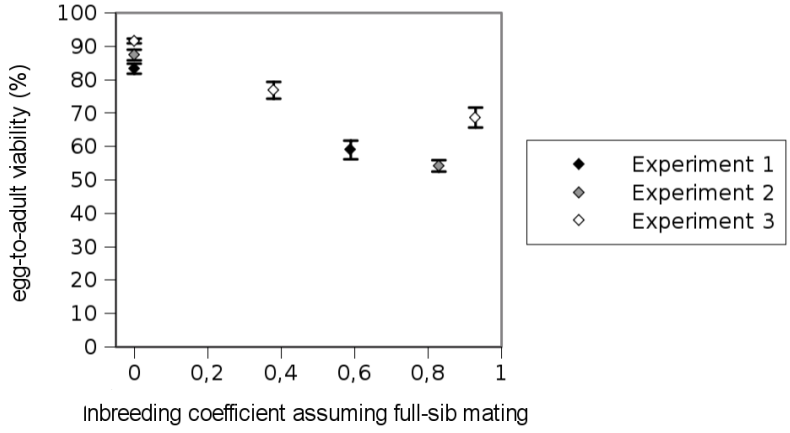

Figure 4: Effect of inbreeding on the egg-to-adult viability (percentage of fertile eggs that survived to adulthood), plotted as a function of maximum inbreeding coefficient. The results stem from three separate experiments, for details see Material and Methods.

The learning performance of the crosses was tested in the assay, for which the results reported above indicated most pronounced inbreeding depression: short-term memory after three conditioning cycles (compare Fig. 2B). Their learning performance in this assay was indistinguishable from that of the outbred population (Fig. 5B). The confidence interval of difference between crosses and outbred is small: (-0.06,0.06). This experiment also confirmed that inbreeding depression for learning performance was weak: the inbred lines had only slightly lower learning scores than the outbred lines and crosses; the difference was only significant if the outbred and crossed treatments were pooled ($F_{1,61}$=4.8, P=0.032). There were also significant differences among the three blocks, in which the experiment was carried out (Anova, $F_{2;61}$=13.2, P <0.0001).

For odor avoidance, crosses between inbred lines did not differ from outbred base population ($F_{1,27}$=0.06, P=0.8; Fig. 3). Both odors were avoided, octanol significantly more than MCH, just as in the other experiments ($F_{1,27}$=17.83, P=0.0002; interaction inbreeding status × odor P=0.6; the interaction was removed from the final model).

3.3 Variation among inbred lines

After 12 generations of inbreeding, we also tested each line separately, to study the variation of learning performance among the lines, and its relationship with egg-to-adult viability. Learning performance turned out to be positively correlated across lines with their egg-to-adult viability (Fig. 6A; Pearson's r=0.63, *d.f.*=12, P=0.015). The inbred lines varied substantially with respect to both learning performance ($F_{13,149}$=3.67, $P < 0.0001$) and egg-to-adult viability ($F_{13,76}$=14.8, $P < 0.0001$). The normal probability plot (Fig. 6B) indicates that the line means of the learning scores fitted the normal distribution almost perfectly (Anderson-Darling normality test, A=0.1083, P=0.99). The corresponding means for viability also did not deviate from normal distribution (A=0.313, P=0.51). Except for one (line 17, t=2.0247, d.f.=8, P=0.077), all the inbred lines had learning scores significantly greater than zero. According to Dunnett's test, only two lines had significantly worse learning scores than the outbred (P<0.05). In contrast, the majority of lines were inferior to the outbred for egg-to-adult viability (P< 0.05). Variance among the lines accounted for 77 % of variance in learning scores and 94 % of variance in egg-to-adult viability values. It should, however, be noted that each replicate was based on 100 individuals, so the within-line among-replicate component underestimates the variation among individual flies within lines. The genetic coefficient of variation (square root of among-line variance divided by mean of the trait, [79]) was 0.68 and 0.82 for learning and viability, respectively. Inbreeding depression could also be calculated for each line separately; the coefficient of variation of this line-specific inbreeding depression (square root of variance among lines divided by the mean inbreeding depression) was 1.01 and 0.54 for learning and viability, respectively.

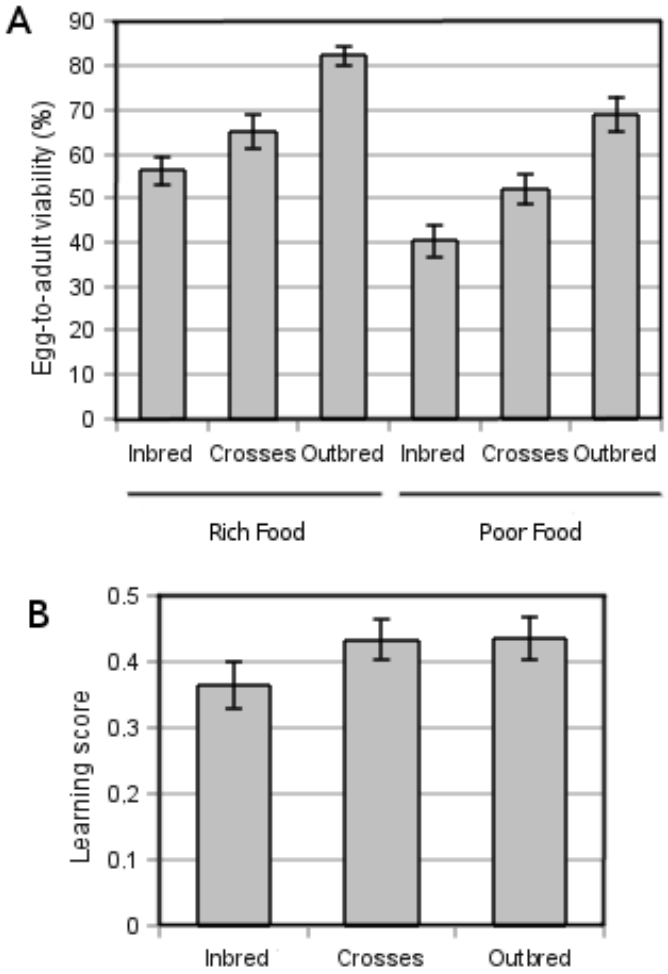

Figure 5: Comparison of strongly inbred lines (12 generations of sib-mating; $0.75<F<0.93$), crosses between inbred lines and outbred base population. (A) Egg-to-adult variability on normal and poor food, $N=12$ vials per food level for inbred, 16-17 per food level for crosses and outbred. (B) Learning ability; $N=21$-22 learning scores per bar.

As an alternative estimator of inbreeding depression, we also calculated the mean and standard error of inbred lines (mean ± standard error of the lines) and outbred base population (learning: inbred lines 0.44 ± 0.02, outbred population 0.54 ± 0.04; viability: inbred lines 51.32 ± 4.9, outbred population 85.29 ± 2.65).

4 Discussion

Our study confirms that inbreeding adversely affects egg-to-adult viability in *Drosophila* [46, 14, 15]. Twelve generations of sib mating reduced viability by about 30 %; the effect tended to be even more pronounced on poor food (40 %). Only 15 of the initial 50 inbred lines survived to the 12th generation and it is probable that the survival of lines was positively correlated with larval viability. It is thus likely that the observed inbreeding depression considerably underestimates the overall effect of inbreeding on viability, even though we did not detect any direct evidence for purging of deleterious alleles (see below). A substantial viability reduction was already observed after two generations of full-sib mating. This confirms results from other studies which demonstrated strong inbreeding depression for fitness-related traits in *Drosophila* (reviewed in [27]).

The quantitative effect of inbreeding on learning performance varied among our experiments. This, together with differences in learning performance observed between blocks within experiments, is consistent with the general observation that behavioral, and in particular cognitive traits are highly labile and sensitive to uncontrollable environmental variation. Nonetheless, all experiments showed at least a tendency for learning performance to be reduced in inbred compared to outbred flies. Because we observed a positive correlation between learning performance and viability across inbred lines, and because many lines were lost in the course of inbreeding, it is possible that with the least viable lines also the lines with the lowest learning performance went extinct. This would have led to an underestimation of the effect of inbreeding on learning, although perhaps to a lower degree than for viability because inadvertent selection during inbreeding may have acted directly on viability but only indirectly (via the positive correlation with viability) on learning. However, the inbreeding depression for learning observed in the additional set of moderately inbred lines (F=0.38) is of similar magnitude as for the highly inbred lines, and none of these lines were lost prior to the learning assays. Taken together, our results indicate a substantial, but not too severe, effect of inbreeding on learning (on average about 18 % in the highly inbred lines). The inbreeding depression for learning performance thus appears to be lower than inbreeding depression for viability observed in the same set of lines. It also seems lower than inbreeding depression for other fitness-related traits, such as number of surviving offspring per female (87 % of inbreeding depression in competitive conditions, and 27 % under uncrowded conditions; [101]), male

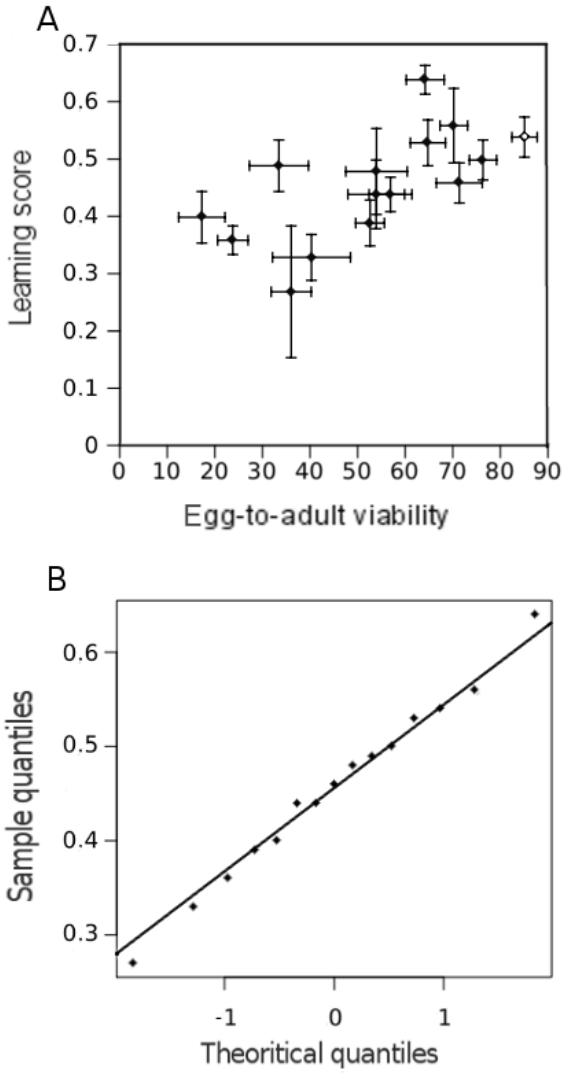

Figure 6: Variation among inbred lines. (A) Mean egg-to-adult viability and short-term memory values of individual inbred lines (filled symbols), compared to the outbred base population (open symbol). Bars indicate one standard error. (B) The normal probability plot of inbred line means of short-term memory learning score; the close correspondence between the predicted and observed quantiles indicates a good fit of line means to the normal distribution.

mating competitive ability (decrease of 5.9 to 10.7% per 10% increase in F; [170], or aberrant courtship pattern [135]. One complicating factor in such comparisons is that the observed homozygosity in the inbred lines may have possibly been lower than expected (expected F=0.75 to 0.93 in our highly inbred lines), because natural selection during the inbreeding process may have favored heterozygous individuals [167]. However, this also applies to other inbreeding studies, so it should not affect the conclusion that learning performance seems less affected by inbreeding than some other fitness-related traits.

Apparently, not all behavioral traits are impaired by inbreeding; in our study the unconditioned responses to odors did not show inbreeding depression. This indicates that cognitive traits differ in their natural genetic variability and/or in their genetic architecture. This result also suggests that this trait, which is also related with several behaviors based on odor perception, may be under particularly strong purifying selection.

Despite the heavy loss of lines in the course of inbreeding, we found no direct evidence that deleterious alleles have been purged during inbreeding, neither for learning nor egg-to-adult viability. In our study, crossing inbred lines restored learning performance to the level of, but not beyond, the performance of the outbred flies, and the viability was intermediate between the inbred and outbred flies. Nonetheless, some purging may have still occurred; purging can be difficult to detect, due to a variety of reasons [11]. According to previous studies, only 20% of mammal species tested and 24% of plants showed purging with very variables ranges [31]. Moreover, purging may vary substantially even among populations of the same species [48, 98]. It has been shown that purging is more efficient in large populations [100, 74, 191] and for alleles with large effects [68]. Deleterious alleles with weak effects are difficult to purge because the effects of genetic drift may outweigh purging selection for these alleles [108, 68]. The only partial restoration of viability in the between-line crosses was not due to fertilization failure (eggs showing no signs of development were eliminated from the assays, see Materials and Methods). However, the parents in the crosses were themselves inbred, so the incomplete restoration of viability in the crosses may have been due to low quality of offspring produced by inbred mothers. Hence, even though other explanations cannot be excluded, this observation may reflect an effect of maternal inbreeding. If so, such maternal inbreeding effect would mask a potential effect of purging of deleterious alleles.

Learning performance varied significantly among inbred lines, with some lines showing the same learning ability as the outbred and some lines showing clear inbreeding depression. Assuming that all inbred lines had increased homozygosity at genes affecting learning, this suggests that homozygosity only on average, but not in all cases leads to reduced learning scores. Hence these results are more consistent with partial dominance rather than overdominance as the main mechanism contributing to inbreeding depression for learning [27]. Furthermore, variation in learning performance among our inbred lines conformed very well

to the normal distribution and even in the worst-performing line the learning score was only reduced by half compared with the outbred flies. This suggests that this variation is due to multiple loci with small effects on learning ability. It is still possible that some alleles causing major learning impairment were lost in the course of inbreeding with the lines that went extinct. However, as discussed above, the crosses between inbred lines provided no evidence of such purging. Furthermore, the additional set of moderately inbred lines, assayed before any line loss, showed a similar degree of inbreeding depression. Thus, even though mutants unable to learn have been identified in laboratory screens [47, 41], such mutants must have been rare or absent in the natural population from which our flies originated. This would indicate that in nature such mutants are strongly selected against, either because strong learning impairment greatly reduces fitness or because such mutants suffer from other deleterious effects.

The fact that on average inbreeding depression for learning is moderate despite large variation among inbred lines suggests that, in the gene pool of the base population, alleles that reduce learning were not exclusively or predominantly recessive. This is consistent with the notion that, within the normal range of variation, learning ability is under stabilizing rather than directional selection. Under directional selection on a quantitative trait, alleles that reduce the trait value are eliminated more readily if they are dominant rather than recessive. Recessive deleterious alleles are thus more likely maintained and at may reach higher frequencies; as a consequence, the standing genetic variation is expected to show directional dominance [109]. In contrast, under stabilizing selection on a polygenic trait, alleles that increase the trait value are as likely to be deleterious as those that decrease the trait value. Hence, which polymorphisms are maintained under stabilizing selection is not affected by the direction of dominance [109, 44], although there may still be some directional dominance for physiological reasons [201].

Selection experiments with *Drosophila* also suggested that learning performance in natural *Drosophila* populations is indeed under stabilizing rather than directional selection (i.e., is optimized rather than maximized). First, learning performance of fruit flies can be readily improved by experimental selection [107, 128, 156, 55]. Second, some selection experiments demonstrated negative genetic correlations between learning ability and other fitness-related traits, such as larval competitive ability, tolerance to chronic malnutrition or lifespan [129, 96, 23]. The resulting evolutionary trade-offs would constrain the evolution of superior learning performance [161].

However, in the present study, learning performance was positively correlated across inbred lines with viability. This suggests that some homozygous allele effects reducing viability had negative pleiotropic effects on learning performance. These might, for instance, be due to alleles involved in some general biological functions; impairing these general functions affects

a multitude of traits, including cognitive ones. As a result, only healthy flies capable of high survival would be good learners. The positive genetic correlation between viability and learning performance stands in contrast to negative genetic correlations between learning and fitness-related traits observed in selection experiments [129, 96]. This apparent contradiction could be in part due to different base populations or different conditions under which viability was assayed (standard food medium here, low food quantity in [129], poor food quality in [96]). However, it could also imply that the response to selection and variation among inbred lines are largely based on different sets of loci. The response to selection for better learning is likely to be based on effects of a few, possibly initially rare alleles, which may improve the trait under selection beyond the average of the population, but which may also show antagonistic pleiotropy. Consistent with this notion, line cross analysis suggests that the response to selection for better learning in Mery & Kawecki's (2002) experiment was based on a few alleles of large effects on learning traits [92]. In contrast, as argued above, variation among our inbred lines seems to reflect cumulative effects of a larger number of loci, most of which do not specifically affect learning, but rather have broad, positively correlated, effects on various aspects of performance, including viability. Other things equal, loci with even allele frequencies are expected to contribute more to variance among inbred lines than loci with skewed allele frequencies [34]. Furthermore, even though additive effects contribute to variation among inbred lines, much of the variation may be due to different numbers of recessive deleterious alleles fixed in different lines. Hence, the positive correlation between learning and viability across inbred lines does not preclude the existence of a trade-off between them.

Only a small number of other studies have investigated inbreeding depression of cognitive functions, most of them finding that these functions are sensitive to inbreeding depression. For instance, spatial learning ability in rats is lower in inbred than in (unrelated) outbred strains [73], and correlative data suggest that inbreeding depression also affects cognitive abilities in humans [12, 3, 166, 1], although not systematically [137]. Human studies are particularly difficult to interpret because socio-economic factors can bias population comparisons. Our experimental approach allowed us to avoid these problems: we could directly compare inbred lines to their ancestral outbred population and eliminate correlation between the degree of inbreeding and the environment. The results indicate that while inbreeding does on average reduce learning ability, the effects are relatively mild and some highly inbred lines learn as well as their outbred relatives. This latter result is important in view of the fact that the vast majority of research on mechanisms of learning in *Drosophila* is carried out on highly inbred strains. From an evolutionary perspective, our study is consistent with the hypothesis that in natural *Drosophila* populations learning is under stabilizing selection, with substantial genetic variation segregating in the population. As already demonstrated in selection experiments, this genetic variation would allow those populations to evolve rapidly

substantially improved learning performance, should the fitness advantage of learning became greater or the trade-offs less important.

Acknowledgements

We thank two anonymous referees for comments on a previous version. This work has been supported by Swiss National Science Foundation grants to Tadeusz J. Kawecki and Christoph R. Haag.

Part III

Natural genetic variability for learning ability, resistance to bacterial infection, and development traits in *Drosophila melanogaster*

Nepoux V., Babin, A., and Kawecki, T.J.

This chapter was a collaborative experiment, done with Aurélie Babin. Both of us contributed to this experiment and equally to its analysis.

Summary

The study of effects of inbreeding on learning ability revealed a positive correlations between this trait and egg-to-adult viablility, which contradicts previous results that showned trade-offs between learning ability and other fitness related traits. Pleiotropic recessive deleterious alleles may be responsible for the positive correlation observed in chapter one. Nevertheless, it may be due to a small number of genes with large effect, hiding other genetic interactions. I derived isofemale lines (F=0.25) from the same natural population. These lines are less adapted to laboratory conditions and were expected to be more representative of the variance of the natural population. They showed similar amount of genetic variability for learning and for three other fitness-related traits possibly related with learning: resistance to bacterial infection, egg-to-adult viability and developmental time. The positive correlation previously observed between learning ability and egg-to-adult viability did not appear in isofemale lines (nor a negative correlation). The hypothesis of pleiotropy cannot be eliminated. Nevertheless, it suggests that the isofemale lines did not fix the highly deleterious pleiotropic alleles possibly responsible for the previous correlation.

1 Introduction

Learning is the ability of an organism to adapt its behaviour to environmental change, in response to past experience. It is widespread across the animal kingdom as most animals are able to learn, even in simple forms. Learning ability plays a fundamental role in the lives of these species [91], but it is associated with two types of costs. First, those related

to physiology: costs of establishing neuronal networks and of memory acquisition [103] may reduce the ability of individuals to face ecological challenges like dessication [130] or pathogens [97, 115, 5]. Secondly, costs can also include evolutionary trade-offs with other fitness-related traits [23, 129, 96]. Learning ability is therefore constrained by complex selective pressures. Evolutionary changes occur on the basis of genetic variation.

Some studies have demonstrated learning ability to be variable in wild populations, using experimental evolution (artificial selection) or inbred lines. Experimental evolution consists of selecting populations with high learning scores. In the honey bee, *Apis mellifera*, and the fruit fly *Drosophila melanogaster*, lines with significantly high learning ability compared to control have been selected [19, 20, 26, 128]. In *Drosophila*, an experiment performed in 2009 by Dunlap and Stephens showed that high and low environmental change rate selected for learning ability, indicating the presence of variability for learning [55]. Variability in learning ability could be maintained by several mechanisms, involving relationship between traits (balancing selection) or not (overdominance). Under certain conditions, antagonisitic pleiotropy can favor balancing selection [164, 75]. Consequently, studying natural genetic variation for learning and its association with other fitness-related traits is necessary to understand how selection can act on populations.

In a previous study, Nepoux and colleagues [138] studied 14 inbred lines randomly derived from a natural base population and subject to 12 generations of sib mating. These lines represented a part of the variation of the base population and had the advantage of being homogenous (within-line variance close to zero). This study found the existence of variability in learning ability. Moreover, we observed a positive correlation between learning ability and another fitness-related trait, egg-to-adult variability. This observation is contradictory to findings from other studies which observed negative genetic correlations between learning ability and fitness-related traits like lifespan or larval competitive ability [129, 23, 96]. The hypothesis proposed to explain the positive relationship between learning and egg-to-adult viability was the presence of pleiotropic genes increasing or decreasing both the traits at the same time. Pleiotropic recessive deleterious alleles may also be responsible for inbreeding depression. Nevertheless, this effect of a possibly small number of genes with large effect may hide other genetic interactions.

However, that previous study explored natural genetic variation in learning and used lines which have been initiated from flies maintained in the laboratory for a few months. It is likely that these flies had adapted to the laboratory, which likely also continued during the inbreeding process. Adaptation to laboratory conditions may change dramatically the genetic variance of a population in three ways. First, laboratory conditions may apply 'new' directional selection leading to a reduction in genetic variation. Second, they may relax or eliminate balancing selection. Finally they may modify genotype x environment interactions,

hence modify reaction norms for a particular trait. Interactions between traits may also been altered.

Like egg-to-adult viability, other fitness-related traits may be involved in genetic correlations with learning ability. It is now well established that immune activity impairs learning score in social insects [5, 115, 158], but this physiological trade-off does not seem to have a genetic basis [6]. A recent study found a contradictory result and reported that a pathogenic infection improves learning score in *Drosophila* (Babin and Kawecki, unpublished data). Several additional assays suggested strongly that this positive relationship may rely, at least partially, on immune activity. In this case, the possibility of a genetic basis for it has to be tested. Note that the fly population used in Babin and Kawecki's study was derived from the same natural base population as the inbred lines obtained in Nepoux and colleagues' study [138].

In this study, we aimed to address the genetic variation in the natural population in learning ability, resistance to bacterial infection and two development traits, egg-to-adult viability and developmental rate. Therefore we collected a representative sample of flies from the same natural population and we measured the traits as soon as possible after sampling [77]. It was done with an isofemale line design, i.e. by rearing the full-sib progeny of a wild female mated with a single wild male [40]. Hence the gene pool of each isofemale line originates from maximum 4 independent haploid genomes (haplotypes) [40]. This design allowed us to ask the following questions: (i) what is the genetic variance for these traits? (ii) are there genetic correlations between them?

What is the genetic variance for learning, resistance to infection and development traits? The amount of genetic variation gives information about the strength of selection that may act on a trait. Recently, Nepoux and colleagues [138] showed that inbred lines harbored genetic variability in learning and egg-to-adult viability. Here we used isofemale lines derived from the same natural population to assess the amount of genetic variability in the natural population they come from. As each line harbours four independent haplotypes, the variance within the isofemale lines will be higher than the variance within inbred lines and still represents a part of the total genetic variance. Among-line variance increases with inbreeding, all things being equal [159, 60] (for a case of dominance, see Fig. 7, for a general case, see fig. 8). As an isofemale line's inbreeding coefficient can be considered equal to 0.25 for lines expanded at a large size [77], their among-line variance should be smaller than for inbred lines. Nevertheless, in the previous study [138], the majority of initial inbred lines have been lost because of inbreeding depression. Some variation, especially rare deleterious alleles, may have been purged during the inbreeding process. Some alleles may also have been lost because of hitchiking, or randomly because of genetic drift, reducing the among inbred-line variance compared to what is expected without them. Consequently, the variance

among the isofemale lines may be higher than the variance among the inbred lines.

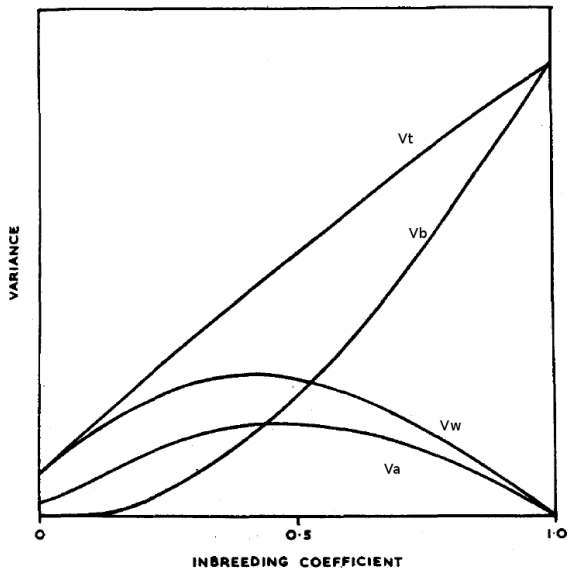

Figure 7: Redistribution of variances with the inbreeding coefficient, for a single fully recessive gene (initial frequency=0.1) [159]. This could represent, without selection, the case of deleterious recessive genes [60]. Vt represents the total genetic variance, Vb the between-line component, Vw the within-line component, and Va the additive genetic variance.

Are there genetic correlations between these traits? Variability among isofemale lines, like in inbred lines, has a genetic basis, even if individuals within a line do not share identical genotypes. It allows testing for correlations between different traits among isofemale lines, whether or not the measurements are performed on the same individuals [40]. A correlation may indicate that the traits are under common genetic basis (pleiotropy), or linkage disequilibrium. Epistasis may also play a part, in association with one of the preceding factors. Therefore, a positive correlation would reveal that the two traits may be under the control of the same pool of genes which act in the same direction. On the opposite, a negative correlation may indicate an evolutionary trade-off between the two traits. It is important to mention here that pleitropy can also occur if no significant correlation is found, when two pools of genes, acting in opposite directions but with the same amplitude work simultaneously which in total cancel the effect.

2 Material and Methods

2.1 Isofemale lines

Lines have been initiated by collecting flies in Valais (Switzerland) in fall 2010 in the natural population from which the inbred lines of the Nepoux and colleagues's study have been

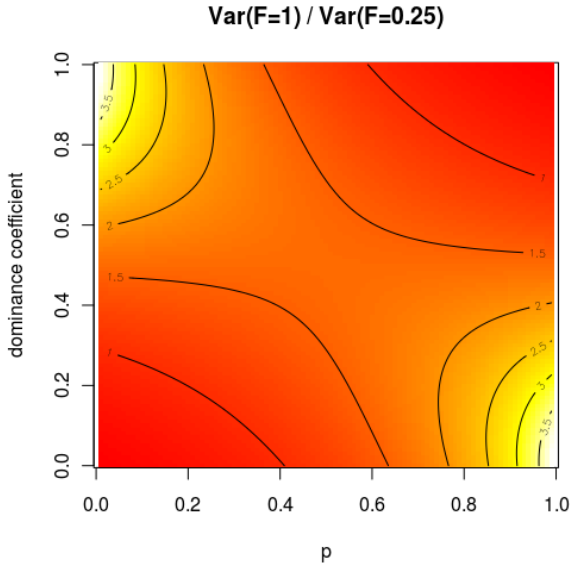

Figure 8: Simulation of the ratio between the genetic variance of two populations (variation arising from one gene and two alleles) with different inbreeding coefficients. The y axis represents the dominance coefficient: 0 means that the heterozygote has the same fitness than the homozygous individual that dispaly the lowest fitness, 0.5 equals to additivity, and 1 means that the heterozygote has the same fitness than the homozygous individual that displays the highest fitness. The x axis represents the initial allelic frequency. In most conditions, the variance of the population with the highest inbreeding coefficient is higher than the other population.

derived in 2007 [138]. Flies were raised on a rich standard food medium containing 8% yeast (David & Clavel, 1965). Flies were maintained under standard laboratory conditions (25 °C, 60% relative humidity, 12:12 L:D cycle).

We initiated a rather high number of lines (∼100). If we assumed that *Drosophila* females mate only once, our lines would be based on 4 haplotypes. However, because *Drosophila* females tend to have multiple matings, our isofemale lines can possibly be based on up to 14 haplotypes assuming up to 6 multiple matings, knowing that microsatellite analyses resulted in the estimation of 4 to 6 matings per female [82]. Taking this into account, we initiated each isofemale line with a pair of wild-collected flies and housed them individually during a week before egg collection [40] to favor the precedence of the sperm of the last mated male [175]. To conserve natural variation and minimize adaptation to laboratory conditions, we expanded the lines at the next generation and tested them at the second and third laboratory generations. From the first generation born in the laboratory, the lines containing less than 10 individuals have been eliminated as they would probably not be able to give sufficient progeny. Eggs were collected from flies of the first generation in the laboratory for development assays (egg-to-adult viability, developmental time), and learning ability and innate preference assays. At generation 2 in the laboratory, eggs were collected to measure resistance to infection at generation 3. At the end, we had 46 to 49 isofemale lines depending on the trait (some of the lines sometimes did not lay enough eggs).

2.2 Trait measurement

2.2.1 Short term memory

Groups of approximately 100 mixed sexes flies aged 3 to 4 days after emergence were assayed in the aversive olfactory conditioning procedure previously described, which is based on the association of an odorant with mechanical shock [130]. Flies were transferred without anaesthesia to test tubes. During the conditioning phase, three conditioning cycles were applied to flies as it has been previously demonstrated to maximise the difference between inbred lines and the natural base population. One conditioning cycle consisted of delivering a first odorant for 30s coupled with six 1s pulses of mechanical shock, followed by a break with humid air for 60s. Then, a second odorant was delivered for 30s without shock, followed by another break with humid air for 60s to complete the cycle. The odorants used to condition flies were octanol (0.6ml/l paraffin oil) and methylcyclohexanol (MCH, 0.6ml/l paraffin oil). Short-term memory retrieval was tested 2 to 6 minutes after the end of the conditioning phase. Flies were loaded in the central chamber of a T-maze and received the two odorants of the conditioning simultaneously, one odorant in each arm of maze. Flies were allowed to move freely and choose one arm of the maze for 1 minute. Flies in each arm of the maze

were counted and learning indexes were computed as the difference between proportions of flies choosing odorant A when B was reinforced with shock and proportions of flies choosing odorant A when A was reinforced with shock. For this calculation, flies which did not choose an odorant and stayed in the central chamber of the maze were excluded. The scale of learning indexes range from -1 to 1, with zero and negative indicating the absence of associative learning processes. Eight replicate scores were obtained for each line with proportions from 8 groups of flies conditioned to avoid MCH and 8 groups conditioned to avoid octanol.

2.2.2 Innate response to odorants

To control for a biased preference for one of the odorants of the conditioning, the innate preference of flies (their preference for the odorants before conditioning, was measured in the T-maze as described above). Two to ten groups of approximately 100 sexes-mixed flies aged 3 to 4 days after emergence were tested per isofemale line. We measured the innate absolute preference i.e. the preference for MCH and octanol when flies were given a choice between the odorant and paraffin oil. Prior to the test, the flies were exposed to the same amount of shock as in a the short term memory test. Proportion of flies choosing the odorant over paraffin oil was used as the response variable for data analysis.

2.2.3 Egg-to-adult viability / developmental rate

Egg-to adult viability and developmental time were assayed on the food medium used to maintain the lines [39] but with only 0.8% of yeast to exacerbate between-line differences. For each line, 200 eggs were placed in a 175-ml bottle with 30ml of food, and the emerging adults have been counted every day until the duration of the emergence period did not exceed the duration of development (i.e. the number between egg collection and adult emergence). Four replicates were set for each line, one replicate (one block) per day. A block effect was hence included in the analysis and could reveal an effect of maternal age. As eggs of all lines could not be collected by a single experimenter, lines were randomly assigned to experimenters on each day. The proportion of adult emergence was used as a measure of egg-to-adult viability. The mean developmental rate of a replicate within a line is 1 over the developmental time and was calculated as follows: $1/(\frac{\sum Nflies*tdays}{Ntotal})$ with Nflies the number of adult emergences counted at time t days (in days from egg collection) and Ntotal the total number of flies that emerged in the vial. In addition, each replicate was checked and quantified for infection symptoms described below and signalling the presence of the natural pathogen (number of spots of black spots on the food medium, numbers of black dead flies and black dead larvae).

2.2.4 Resistance to bacterial infection

Groups of 30 females aged 2 to 3 days post-emergence were collected under CO2 anaesthesia for each isofemale line and let to recover for 24 hours on fresh standard food. The bacterial entomopathogen *Pseudomonas entomophila* [188] was grown at 28°C in standard Luria-Bertani medium (10g Bactotryptone, 5g Bactoyeast, 10g Nacl; 1000mL distilled water). A bacterial pellet was collected after centrifugation (3000rpm, 20min at 4°C) and optical density at 600nm was adjusted to OD600nm$\sim$200 ($\sim$1011 cells per mL) with sterile 0.9% saline solution. Two bacterial concentrations were then used to challenge flies, 1/4 (high concentration) and 1/10 (low concentration) of OD600nm$\sim$200. Groups of flies were inoculated under CO2 anaesthesia by pricking the fly thorax with a needle dipped into the bacterial solution. Flies were transferred back on standard food and maintained under standard laboratory conditions afterwards. Four replicates of 30 females per isofemale line were pricked for each bacterial concentration, one replicate per concentration per line being pricked each day. To control for mortality induced by pricking itself, a control group of 30 females per line was pricked with a needle dipped into 0.9% saline solution. As both sexing and pricking steps were performed under CO2 anaesthesia, each step was performed by a different experimenter to buffer variation in fly survival induced by differences in experimenter speed and hence variation in CO2 exposure. Dead flies were counted for 60 hours three times a day at precise time points, and final survival fraction was used as variable in the analysis.

2.3 Natural infection with an unknown pathogen

Some of the isofemale lines turned out to be infected with a pathogen before we brought them to the laboratory. The infection symptoms were the following: reduced egg-to-adult viability (up to 40%), non negligible adult and larval mortality, melanisation of dead larvae and pupae, and development of black spot on the food medium sometimes around dead flies. These symptoms were weaker in flies raised on rich food, and the infection seemed to be transmitted from one generation to another. We experimentally infected flies caught in Valais (Switzerland) in fall 2007, with residues from sick individuals of other strains, and treated them with antimicrobial compounds. Groups of 80 or 100 eggs were placed onto food with the antimicrobial compound tetracycline (0.25mg/mL) for antimicrobial treatment, or regular food for controls. Then, eggs were soaked with a suspension of homogenized melanised dead larvae, pupae and black spots on food for infection treatment or soaked with water for controls. 6mL/L [96]. The number of pupae in each infection treatment and antimicrobial treatment were counted as a measure of egg-to-adult viability. At the same time, the antibiotic tetracycline (0.25mg/mL) as well as the antimicrobial kanamycin (0.2mg/mL), streptomycin (0.2mg/mL) and the anti-fungal propionic acid (6mL/1L water

in food [96] were tested on two other different fly lines not used in the following assays but that showed identical symptoms: one inbred line cited above, and one stock line in our laboratory. One to four replicates were tested for control treatment, and two to four replicates for infection treatment.

2.4 Data analysis

Egg-to-adult viability upon infection with the unknown natural pathogen was analysed with non-parametric Mann-Whitney and Kruskal-Wallis tests.

Short-term memory data, innate preference data, resistance to infection data and egg-to-adult viability data were analysed by fitting a linear mixed effect model with the REML method in JMP® 8.0.1. Isofemale line was included as random effect, and block (day, it had only 4 levels maximum), experimenter and odorant (for innate preference data only) as fixed effects. As some of the lines showed infection symptoms, numbers of black spots on food, black dead larvae, and black dead adults were included as co-variables in the analysis of egg-to-adult viability and developmental rate. Because symptoms were quantified only in the development assay, a line-specific mean of symptoms was used as co-variable for the analysis of learning data and survival of infection data. Residuals of the models were tested for the ANOVA assumptions.

We estimated the genetic variance of each trait from the results of the REML models following [77]. As all fly lines were reared under similar laboratory conditions, we can expect that the among-line variance (effect of isofemale line in the model) is mostly of genetic origin. In this case, the among-line variance would give a rough estimate of the genetic variance (V_G) of the natural population we sampled, assuming that sampling was large enough to be representative. However, as each line was founded with 4 independent haplotypes, within-line variance also contains genetic variance, although a smaller amount. Consequently the among-line variance under-estimates the total genetic variance of the line. Hoffmann and Parsons [77] went further in the analysis and proposed formulae to calculate the additive genetic variance from the between-line ($V_B = (3/4)V_A$) and within-line ($V_W = V_A/2$) variance components. The formulae are based on several assumptions (infinite size of the line population, no epistasis), likely unrealistic.

As most of the observed variance is genetic, we calculated genetic correlation coefficients between traits. Significance of the correlations was calculated with Pearson's correlation tests. P-values were corrected for multiple tests (Bonferoni correction, n=6) which lowered the significance threshold to 0.05/6=0.008.

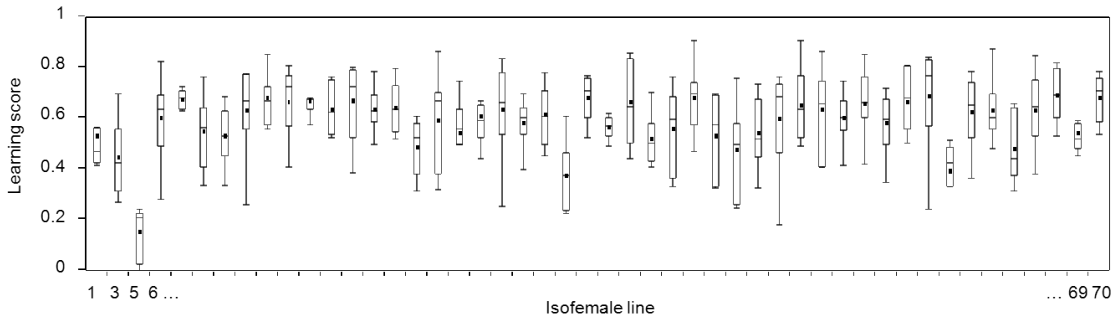

Figure 9: Natural variability in learning score measured in 48 isofemale lines. Boxplots represent the dispersion of replicates around the median and the black bold point is the mean line phenotype.

Table 1: Variance components of random effects (± s.e.) for short-term memory (Learning), innate absolute preference (Preference), resistance to infection (Resistance), egg-to-adult viability (Viability) and developmental rate (Development).

Source (mean ± s.e.)	Learning	Preference	Resistance	Viability	Development
Among-line variance	0.0079	0.0012	0.0044	0.0044	0.0000042
	± 0.002	± 0.0004	± 0.003	± 0.001	± 0.000001
Residual	0.0180	0.0127	0.0284	0.0090	0.0000100
	± 0.026	± 0.0006	± 0.004	± 0.001	± 0.00001

3 Results

3.1 Genetic variance of traits

3.1.1 Short-term memory

Learning score of all lines was to some extent superior to zero (mean 95% CI did not include zero), meaning that all lines were able to learn to some degree in our conditioning procedure (Fig. 9). For this trait, about one third of the variance of random effects was explained by among-line variance (LR-χ^2 = 314, d.f.=1, p<0.0001; Tab. 1). Short-term memory did not co-vary with the mean symptoms of the natural infection ($F_{1,45}$=0.6, p=0.4). The block effect was not significant ($F_{2,298}$=1.8, p=0.2).

3.1.2 Innate response to odorants

Flies showed aversion to both odorants used in the conditioning procedure (mean ± s.e.: MCH 0.36 ± 0.01, octanol 0.29 ± 0.01; t test, comparison with theoretical mean: MCH, t=20.6, p<0.0001, octanol, t=33.6, p<0.0001). A small amount of random effects variance was accounted for by among-line variance in fly innate preference (LR-χ^2 = 980, d.f.=1, p<0.0001; Fig. 10, Tab. 1). Block (day) had a significant effect on innate preference ($F_{3,617}$=14,

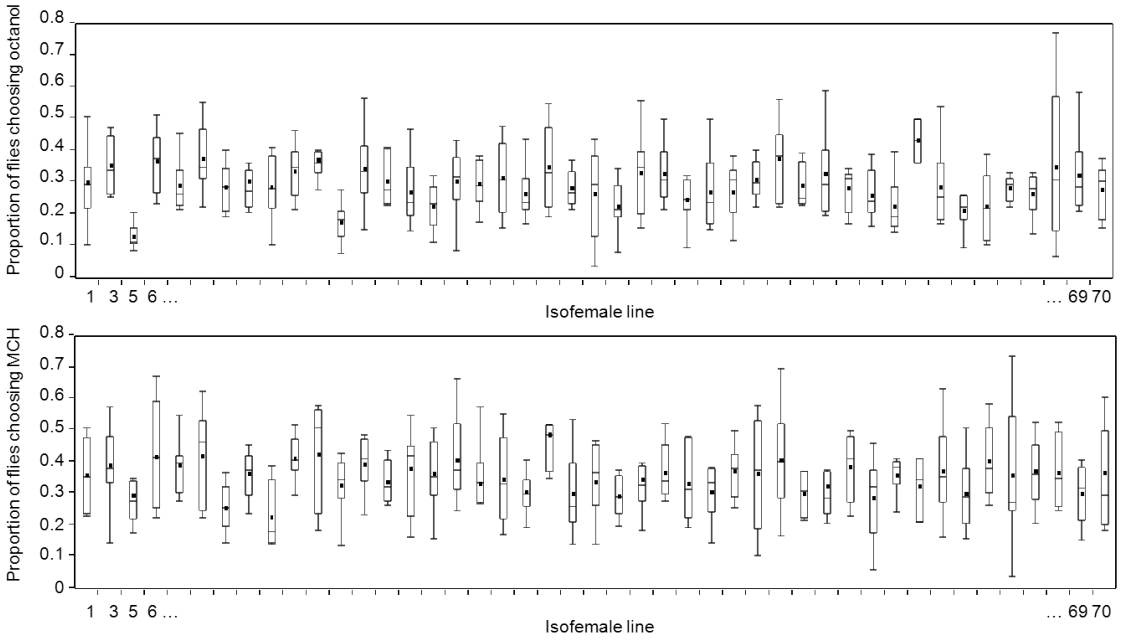

Figure 10: Natural variability in innate absolute preference for MCH (top graph) and octanol (bottom graph) measured in 44 isofemale lines. Boxplots represent the dispersion of replicates around the median and the black bold point is the mean line phenotype.

$p<0.0001$), as well as the odorant offered against paraffin oil ($F_{1,608}=94$, $p<0.0001$). As for short-term memory, innate absolute preference did no co-vary with the mean symptom of natural infection ($F_{1,43}=0.3$, $p=0.6$).

3.1.3 Egg-to-adult viability / developmental rate

Egg-to adult viability varied mainly because of among-isofemale line variation (LR-$\chi^2 = 264$, d.f.=1, $p<0.0001$; Fig.11). The effect was strong as among-line variance explained one third of the random effects variance (Tab. 1) However, egg-to-adult viability depended on the experimenter who collected eggs ($F_{4,154}=2.6$, $p=0.04$), and not surprisingly co-varied with symptoms of the natural infection (black spots on food: $F_{1,171}=29.3$, $p<0.0001$; melanised dead flies: $F_{1,174}=4.8$, $p=0.03$; melanised dead larvae: $F_{1,173}=0.89$, $p=0.3$). Average infection symptoms were significantly negatively correlated with egg-to-adult viability ($F_{1,43}=21.4$, $r=-0.56$, $p<0.001$; Fig.12).

Very similar results were obtained in the analysis of developmental rate data. Isofemale lines varied in their developmental rate (LR-$\chi^2 = 1526$, d.f.=1, $p<0.0001$; Fig.11). Similary to egg-to-adult viability, among-line variance was equal to about one third of the variance of random effects (Tab. 1). Unlike egg-to-adult viability, developmental rate was not influenced by natural infection with the unknown pathogen (black spots on food: $p=0.4$; melanised dead flies: $p=0.7$; melanised dead larvae: $p=0.7$). The experimenter who collected the eggs

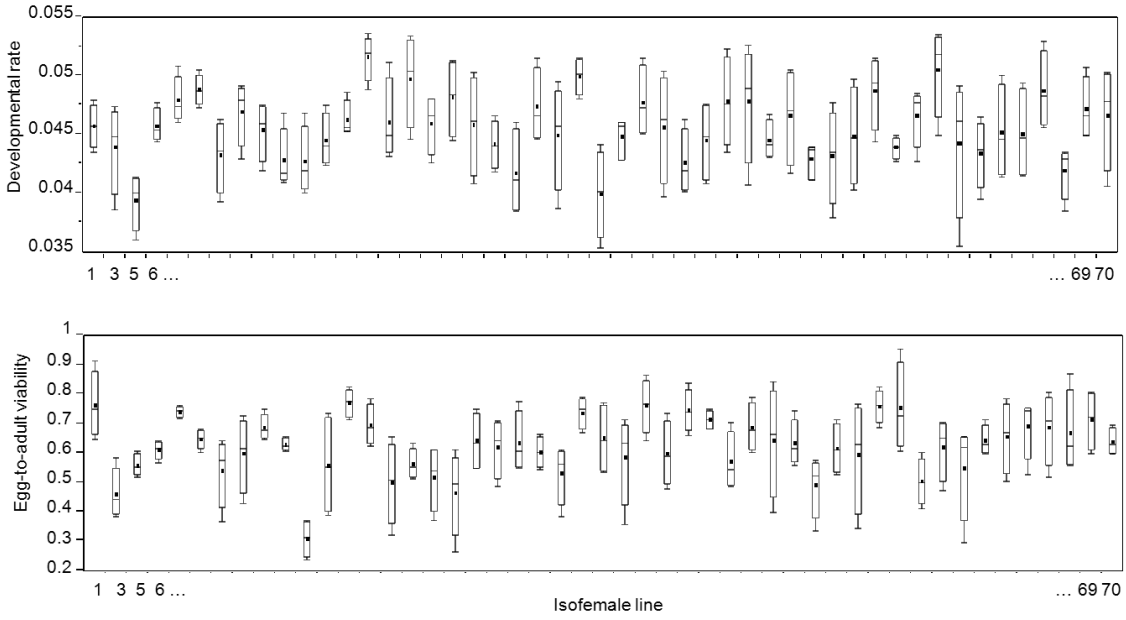

Figure 11: Natural variability in egg-to-adult viability and developmental rate measured in 49 isofemale lines. Boxplots represent the dispersion of replicates around the median and the black bold point is the mean line phenotype.

also affected developmental rate ($F_{4,156}$=2.9 p=0.02).

3.1.4 Resistance to bacterial infection

Both the low and the high doses of bacteria reduced fly survival. As expected, the high dose led to a stronger reduction in fly survival than the low dose (paired Student t test, t=4.1, d.f.=179, p<0.0001), and survival rate with the high dose was positively correlated with survival rate with the low dose across lines (r=0.12, p<0.0001). From then on, we did further analysis on survival data of the high dose of bacteria only as it magnified line differences. The analysis of co-variance revealed a significant but small isofemale line effect ($\sim$1/6 of random effects variance; LR-χ^2 = 71, d.f.=1, p<0.0001; Fig.13, Tab. 1). There was a significant effect of the experimenter on the resistance to bacterial infection ($F_{7,150}$=9.7, p<0.0001). Resistance to bacterial infection co-varied positively with the average symptom of natural infection ($F_{1,44}$=19.4, p<0.0001, correlation see Fig.14).

3.2 Genetic correlations between traits

After Bonferoni correction for multiple tests on the significance threshold, we did not detect significant genetic correlations between traits (Tab. 2, Fig. 15). Nevertheless, with the significance threshold of 0.008, the negative correlation between resistance to infection and egg-to-adult viability was marginally significant ($F_{1,42}$=5.6, p=0.02, r=-0.35; Fig. 15). This

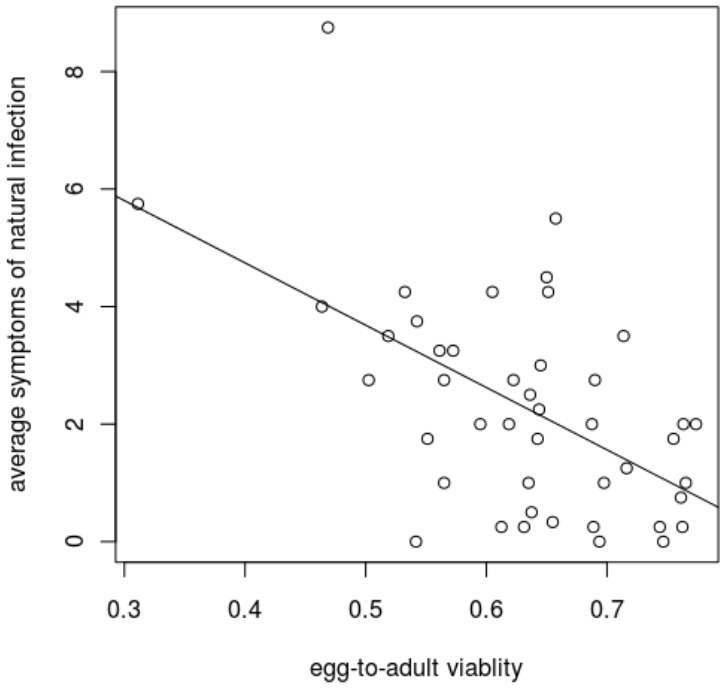

Figure 12: Correlation between egg-to-adult viability (proportion) and average symptoms of natural infection. The black lines corresponds to the regression lines

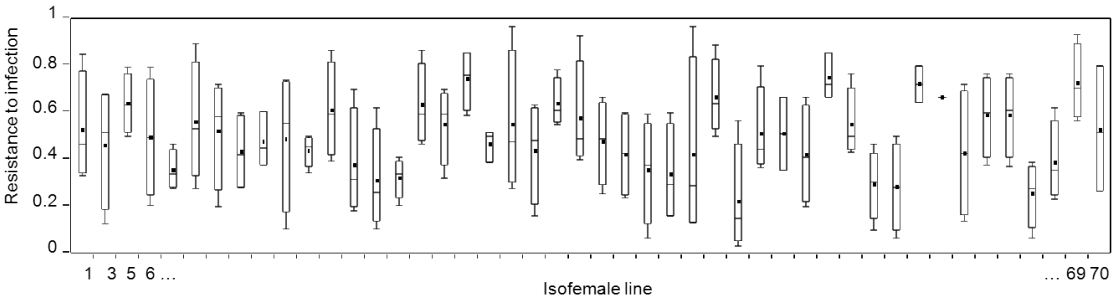

Figure 13: Natural variation in resistance to infection with the high dose of bacteria measured in 46 isofemale lines. Boxplots represent the dispersion of replicates around the median while the black bold point is the mean line phenotype.

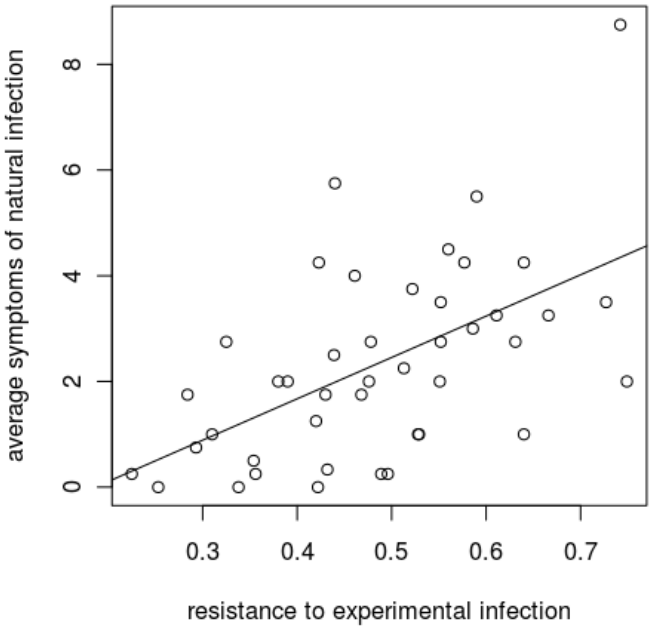

Figure 14: Correlation between resistance to experimental infection (proportion) and average symptoms of natural infection (mean of individuals dead with symptoms during the egg-to-viability experiment).

Table 2: Coefficients of correlation across isofemale lines between learning score (Learning), resistance to infection (Resistance), egg-to-adult viability (Viability) and developmental rate (Development) across lines.

	Learning	Resistance	Viability	Development
Learning	-	r=-0.26 ; p=0.09	r=0.24 ; p=0.11	r=0.23 ; p=0.13
Resistance	-	-	r=-0.34 ; p=0.02	r=-0.01 ; p=0.93
Viability	-	-	-	r=0.28 ; p=0.07
Development	-	-	-	-

correlation became non significant when we included the symptoms of natural infection as co-variable and calculated a partial correlation ($F_{1,41}$=0.3, p=0.06). Innate absolute preferences for MCH and octanol were not correlated with learning score, which means that a bias in odorant preference prior to conditioning did not add variation in learning scores.

3.3 Natural infection with an unknown pathogen

Infection with the natural pathogen reduced the proportion of larvae that reached pupation (Mann-Whitney, χ^2=4.5, d.f.=1, p=0.03; Fig. 16).

Treatments applied to the two other lines which exhibited disease symptoms resulted in divergent patterns. The stock line showed a slight reduction in egg-to-adult viability upon anti-microbial treatment (Mann-Whitney, χ^2=8.9, d.f.=3, p=0.03; Fig. 17) while the egg-

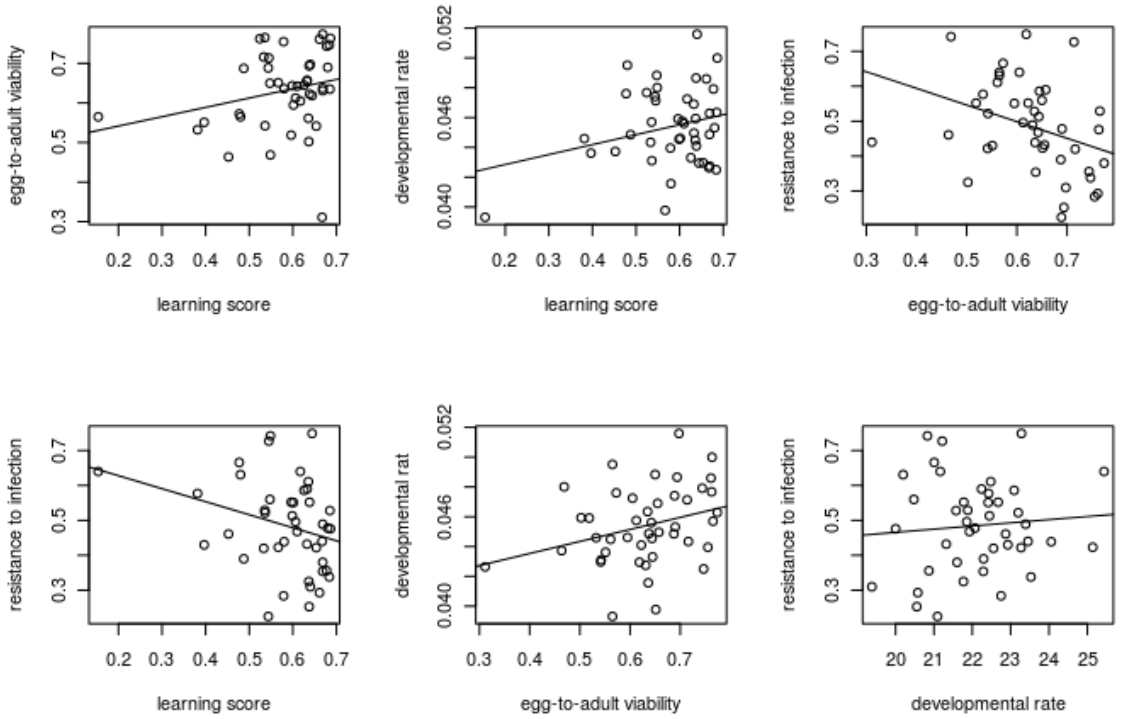

Figure 15: Genetic correlations between traits. Black lines correspond to regression line.

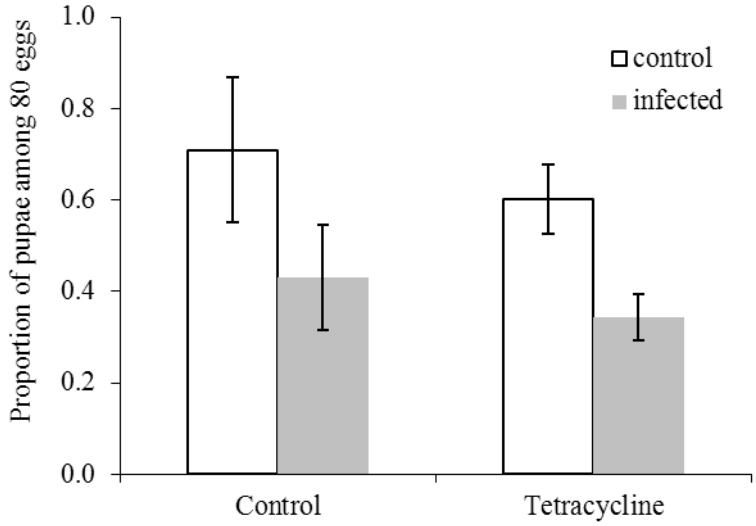

Figure 16: Egg-to-adult viability upon infection with the natural pathogen. Valais 2007 flies were infected and treated with tetracycline (0.25mg/mL). White bars correspond to the control uninfected treatment, while grey bars correspond to the infected treatment.

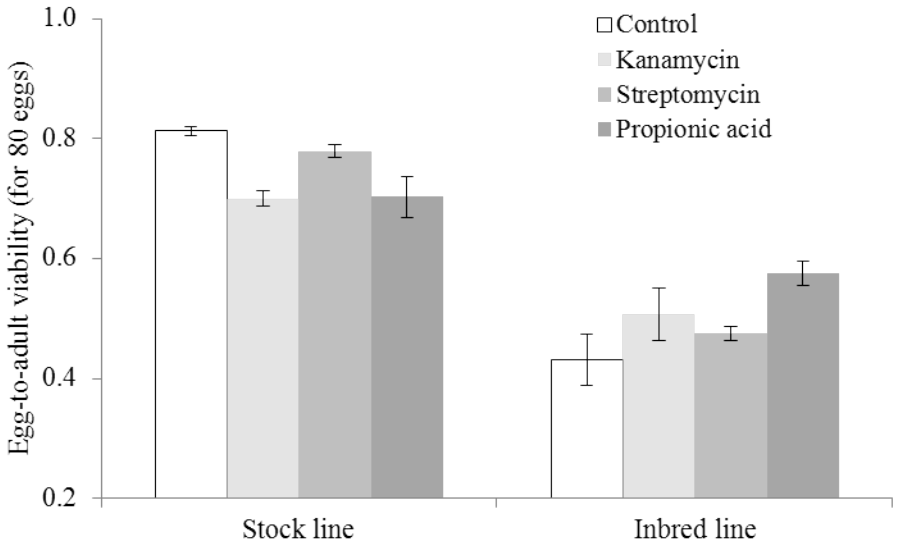

Figure 17: Egg-to-adult viability in a stock line and an inbred line which showed the symptoms of natural infection. Control uninfected treatment is in white, Kanamycin (0.2ml/mL) in very light grey, Streptomycin (0.2ml/mL) in light grey, and Propionic acid (6mL in 1L water) in dark grey.

to-adult viability of the inbred line tended to be slightly improved (Mann-Whitney, χ^2=6.8, d.f.=3, p=0.08; Fig. 17).

4 Discussion

Genetic variation of measured traits

Variance among lines was significant and explained relatively large amounts of variance for development traits and learning ability. It indicates that the natural base population we sampled harbours some genetic variation for these traits, and hence has the potential to evolve under natural selection. By contrast, smaller amounts of variance were explained by among-line differences for resistance to infection and innate response to odorants. Not surprisingly, the major part of the variance of random effects was accounted for by variance of residuals (up to about 75%). This variance includes the environmental variance as well as the within-line genetic variance (our lines contained maximum 4 haplotypes).

The learning ability, the learning ability (mean=0.58, variance=0.008), the resistance to infection (mean=0.48, variance=0.004) and the egg-to-adult viability (mean=0.63, variance=0.004) exhibits comparable amounts of among-line variance, which suggests that they harbour comparable amounts of genetic variability. This contrasts with what has been previously observed in inbred lines derived from the same natural population [138], which conclude that learning

ability was less variable, and therefore under stronger selection in nature than egg-to-adult viablity. Developemental rate (mean=0.045, variance=0.000004) varies much less than the variance of other traits, suggesting that this trait is less genetically variable in nature, maybe under stronger selection.

If we compare in more details the results of the isofemale lines and those of inbred lines, obtained in different experiments, we can see that the grand mean learning score of the isofemale lines is higher than the mean score of the inbred lines (mean=0.58, among-line variance=0.008, for isofemale lines and mean=0.45, among-line variance=0.006 for inbred lines). Differences in means could reflect inbreeding depression. The purging in inbred lines during the inbreeding process could have led to a reduction of variance among inbred lines compared to what is expected in the absence of natural selection, i.e a complete representation of the variability of the natural base population [60, 159]. However it is hazardous to compare inbred lines and isofemale lines which were initiated from the same base natural population but with a 4-year time interval. Noise in the total variance may have been induced by accidental variation in experimental conditions, and adaptation to laboratory conditions occurred for inbred lines. The selection pressures applying on the natural population may also vary over time. Some studies showed stability of populations over time for some morphological traits, but it has also been argued that some populations of *Drosophila* were quite unstable [88].

We should also interpret our results with caution as some isofemale lines were naturally infected with an unidentified pathogen. We showed in an independent experiment that this natural pathogen reduced egg-to-adult viability. So unsurprisingly we found that two major symptoms of this infection had a significant negative effect on egg-to-adult viability of isofemale lines. The more numerous the signs of infection were, the lower the proportion of emerged adults was. Developmental rate was nearly not affected by the natural infection suggesting that flies that succeeded in coping with the infection had a quite normal development. On the opposite, resistance to experimental infection co-varied positively with the number of symptoms. It supports the idea that exposition to the natural pathogen already elicited an immune response which provided flies with a long-lasting protection against subsequent infection. As outliers, short-term memory and innate absolute preference were not influenced by the presence of the natural pathogen. This could possibly lead to expect no relationship between resistance to infection and learning ability.

Genetic correlations between traits

Learning ability and development traits Short-term memory and egg-to-adult viability were not significantly correlated nor were short-term memory and developmental rate. This does not confirm what was previously found by Nepoux and colleagues [138] in which, inbred

lines showed a positive genetic correlation between learning score measured as short-term memory and egg-to-adult viability. However, it clearly indicates that there is no genetic trade-off between the two traits. The non-significant correlation we observed does not exclude the hypothesis of a pleiotropic relationship between the two traits. The effect of genes causing the increase of both traits could be cancelled by the effect of other genes acting in the opposite direction. Moreover recessive pleiotropic highly deleterious alleles, counter-selected in nature, are probably rare in the natural population. It is therefore unlikely to find them at an homozygous state in isofemale lines, which would mean that the two parents carried these alleles and randomly met. Consequently, the expression of these alleles would be reduced in isofemale lines compared to inbred lines. This would flatten the scatter plot, and lead to a diminution or elimination of the correlation.

Learning and resistance to infection Learning score was not significantly correlated to resistance to infection. This result suggests that the learning improvement recently observed in infected fruit flies (Babin and Kawecki, unpublished data) may not have a genetic basis. It further suggests that if this learning improvement is due to immune activity, it might rely on either a physiological trade-off or on bi-directional communication between the nervous system and the immune system (Babin and Kawecki, unpublished data). The absence of genetic correlation goes in the same direction as previous studies that did not suspect an evolutionary basis for the physiological trade-off between learning ability and immune activity detected in social bees [6]. A similar study in *Drosophila* based on artificial selection also did not find an evolutionary trade-off between resistance to parasitoids and olfactory learning ability [97]. As for learning ability and development traits, the absence of correlation does not necessarily imply the absence of pleiotropic interactions between learning ability and resistance to infection. Although the natural infection did affect resistance to infection, it is not likely to interfere in the correlation with learning ability as short-term memory was not affected by it.

Resistance to infection and development traits We found a marginally significant negative correlation between resistance to infection and egg-to-adult viability but not between resistance to infection and developmental rate. This could indicate the existence of a physiological trade-off between resistance to infection and egg-to-adult viability. At the genetic level, this could be supported by antagonist pleiotropic effects of resistance to infection and egg-to-adult viability genes, and epistasis or linkage disequilibrium. Our finding would be consistent with the literature where *Drosophila* exhibit smaller sizes at emergence when they succeeded in defending themselves against parasitoid attacks [62], and moth lines selected for viral resistance have slower development and higher failure probability for egg development [18]. In nature, evolutionary trade-offs between traits is a major cause of maintenance of

genetic variation. This could explain the high levels of genetic variability we observed in the two traits. However we have to mitigate our results because of the occurrence of the natural infection. Some of the lines carried a pathogenic infection that elicited strong symptoms under laboratory conditions, and the expression of infection symptoms was negatively correlated with egg-to-adult viability and positively correlated with resistance to infection. When considering these elements, the observed negative relationship between resistance to infection and egg-to-adult viability may simply mean that the isofemale lines which suffered from a stronger natural infection were selected for resistance to pathogens, which gave them an advantage when coping with the experimental infection compared to isofemale lines which were only slightly affected by the natural infection. Alternatively, it is possible that this relationship was due to a long-lasting immune protection in lines infected with the natural pathogen. This is supported by the absence of correlation when natural infection was included in the linear regression model. In this context, the absence of relationship between resistance to infection and developmental rate was not surprising as this trait related to development was not affected by the natural infection.

Egg-to-adult viability and developmental rate For development traits, we found no significant relationship after Bonferoni correction. As for other non-significant correlations, this does not exclude the existence of pleiotropic interactions between the two traits. Nevertheless, as the measurments for egg-to-adult viability and developmental rate have been performed on the same vials, the correlation may be contaminated by covariance of vial effects.

Conclusion With the isofemale line design, we were able to determine, although roughly, the amount of natural genetic variability occuring in a natural population that has been sampled for several studies. The results were consistent with previous work on genetic variation and showed that this population harbours some genetic variability for learning ability, innate preference for odorants, resistance to infection and development traits. Our estimates of genetic variability were however under-estimated as the amount of genetic variability that remained within the lines because of the four founding haplotypes was ignored. Unexpectedly we did not detect any significant genetic correlation between these traits. This may indicate that a previous positive correlation between learning ability and egg-to-adult viability observed in inbred lines from the same population was mostly due to inbreeding depression, even if we cannot eliminate the hypothesis that the traits are under the control of pleiotropic genes. The relationship between these traits may be more complex than what was suggested by the study of inbred lines. To conclude, our study supports that learning ability, resistance to infection and development traits still exhibit genetic variation in the wild, and are hence subject to natural selection. But, from this study, it seems that the maintenance of nat-

ural genetic variability for learning ability cannot be explained by evolutionnary trade-offs between learning ability and the fitness-related traits we tested.

Part IV

Quantitative genetics of learning ability and resistance to bacterial infection in *Drosophila melanogaster*

Nepoux, V., Babin, A., Le Rouzic, A. and Kawecki T. J.

This chapter was a collaborative experiment, done with Aurélie Babin (both of us contributed equally to this experiment and its analysis) and Arnaud Le Rouzic (Chargé de Recherche, CNRS, Gif-sur-Yvette), who performed the statistical analysis.

Summary

The study of effects of inbreeding on learning ability revealed a positive correlations between this trait and egg-to-adult viablility. This may not apply to their additive effects, which are more relevant for evolution. A diallel cross between all the remaining inbred lines tested in part one has been performed in order to investigate the relative amount of nuclear (additive and non-additive effects) and extra-nuclear (maternal and paternal effect) components of variance in learning ability and other fitness-related traits. The nuclear additive genetic variance was higher than other components for learning ability and survival to learning ability, but in contrast, the contribution of maternal effects was most important for developmental traits (egg-to-adult viability and developmental time). This suggests that maternal effects, which reflect effects from mitochondrial DNA, epigenetic effects, or the amount of nutrients that are invested by the mother in the egg, are crucial in the early stage of life, and less at the adult stage. There was no additive genetic correlation between learning ability and other traits. The hypothesis of pleiotropy cannot be eliminated, but these results suggest a different explanation of the positive correlation observed in chapter one between learning ability and egg-to-adult viability. Inbreeding depression for learning ability and egg-to-adult viability were not significant, which contradicts results found in chapter one. A significant inbreeding depression for developmental time and resistance to infection has been found. This could be due to recessive deleterious alleles homozygous in the inbred lines.

1 Introduction

Learning is the ability of an individual to adjust its behavior in response to environmental change [157, 143]. Nevertheless, not all environments select for learning ability [145, 54, 35]. If environmental changes happen too slowly, an individual may not experience them within its lifetime. On the opposite, if changes are too rapid, experience of a set of environmental conditions may even not happen twice within an individual's lifetime. This would make learning ability useless to predict for example the association between signal and reward. In *Drosophila,* it has been experimentally shown that not all environmental changes favour learning ability [55]. Consequently, unstable environment balancing between favourable and unfavourable conditions for learning ability may maintain genetic variation for this trait. Another mechanism possibly favours maintenance of genetic variation: antagonistic pleiotropy, that results in evolutionnary trade-offs [165, 164]. Such trade-offs between learning ability and other fitness-related traits such as ageing [23], or larval competitive ability [129] have previously been demonstrated in *Drosophila.*

By contrast with these findings, a recent study found a positive genetic correlation between learning ability and egg-to-adult viability in inbred lines derived from a natural outbred base population [138]. It indicated that there is genetic variation for learning in the wild. It also suggested that much of this genetic variation is due to pleiotropic recessive deleterious effects of genes affecting functions related to survival. This positive correlation does not need to apply to the effects of alleles expressed in an outbred background, their additive effects.

The genetic variance of traits can be separated into different components: additive genetic variance, dominance and epistasis. Additive genetic variance is the most relevant component for evolution because it can respond directly to selection [63]. Knowing the contribution of the additive genetic variance in the genotypic variance is of crucial importance to determine to what extent a trait can evolve. Narrow-sense heritability, defined as the additive variance divided by the total phenotypic variance [60], or evolvability [79], the additive genetic variance relative to the mean of the trait, are commonly calculated to predict the response of a population to selection. Therefore, concerning the positive correlation previously observed between learning ability and egg-to-adult viability in inbred lines described in [138], estimating the breeding value of each inbred line, i.e. the average performance of a line when crossed with others would allow us to understand whether the positive correlation between these traits was mostly due to inbreeding or additive genetic effects.

Moreover, there is evidence that learning ability may also be involved in physiological trade-offs with fitness-related traits. Learning ability requires energy and resources [103] that are then not available to other physiological functions (e.g. [130]). The same way, other physiological functions may affect the expression of learning ability. This is in particular the case for immune activity in honey bees [115] and bumble bees [158, 5], which impairs

the performance at associating an odorant with a sugar reward. Genetic trade-offs often reflect physiological trade-offs [182]. However, despite the results at the physiological level, the studies so far have not found evidence for a genetic trade-off between learning ability and resistance to parasitoids in *Drosophila* [97] or antibacterial immune activity in bees [6]. Additive genetic correlations would be a powerful tool to detect pleiotropic effects linking learning ability and immunity. Note that the absence of relationship at the genetic level does not mean that there is no pleiotropy if two pools of genes act on both traits, in opposite directions but with the same effect size [60].

In this study, we addressed four questions about the genetic variation of learning ability, resistance to infection and two fitness-related development traits (egg-to-adult viability and developmental time): (i) how large are the additive and maternal/paternal contributions in the observed variation? (ii) are there correlations of breeding values between the traits? (iii) what is the relationship between the phenotype of inbred lines and their breeding values for these traits? (iv) Are the traits we measured affected by inbreeding?

How large are the nuclear (additive) and extra-nuclear (maternal/paternal) contributions in the observed variance? The analysis of the progeny of crosses between inbred lines derived from a natural population allows us to estimate genetic variance components of this natural population. The method developped by Sprague and Tatum defined [180]: (i) the general combining ability of each line (GCA), i.e. half its breeding value [198, 60] and (ii) for each cross, the deviation between the observed phenotypic value and the phenotypic value expected from the breeding values of the parental lines; this deviation corresponds to the specific combining ability (SCA).

Assuming complete inbreeding, GCA and SCA variances correspond to [60, 109]:

$$\sigma^2_{GCA} = \frac{\sigma^2_A}{2} + \frac{\sigma^2_{AA}}{4} + ...$$

$$\sigma^2_{SCA} = \sigma^2_D + \frac{\sigma^2_{AA}}{2} + \sigma^2_{AD} + \sigma^2_{DD} + ...$$

GCA variance is not a direct estimate of additive genetic variance even with completely inbred lines, because this variance includes additive genetic variance (σ^2_A) plus additive-by-additive epistatic variance (σ^2_{AA}) [60, 109] (which can be estimated with the phenotypic values of the F2 progeny of crosses between inbred lines). To make things simpler, epistasis can be ignored [199], but in this case, heritability and evolvability are under-estimated. Similarly, additive-by-dominance (σ^2_{AD}) and dominance-by-dominance (σ^2_{DD}) epistasis contribute to SCA variance, in addition to dominance variance (σ^2_D). However, we can expect the absence

of overdominance, which is very rare in natural systems [138].

Moreover, GCA also includes maternal and paternal extra-nuclear genetic effects that we would like to quantify. Consequently, we used Cockerham and Weir's model [30]:

$$X_{ijk} = \mu + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk}$$

where X_{ijk} is the value of replicate k of the cross between mother line i and father line j, n_i and n_j stands for maternal and paternal nuclear effects respectively, t_{ij} for the interaction between nuclear effects, $m_i$for maternal extra-nuclear effects and p_j for paternal extra-nuclear effects. The traditional estimation of breeding values rely on the estimation of GCA and is $GCA_i = n_i + m_i$ for the mother line, $GCA_j = n_j + p_j$ for the father line. Here we estimated them with $n_i + n_j$ meaning that our breeding values represent only additive nuclear parental effects. Nevertheless, maternal extra-nuclear effects also contain genetic effects (mitochondrial DNA) and non-genetic effects (epigenetic) which can contribue to heritability along with additive nuclear genetic effects. If these effects were large, they would need to be taken into account in order to avoid under-estimating heritability and thus under-estimating evolvability of the traits.

Are there additive genetic correlations (correlations of breeding values) between these traits? We calculated the correlations of breeding values between two traits (r)[60], as an estimation of additive genetic correlations (r_A). "The genetic correlation expresses the extent to which two measurements reflect what is genetically the same character" [60]. A significant correlation would then indicate that the two traits are influenced by the same genes (pleiotropy), or that there is some linkage disequilibrium between them [99]. Nevertheless, the absence of correlation does not mean the absence of pleiotropy if two pools of genes interact and have opposite effects of the same size on both traits [60]. Concerning the relationship between learning ability and egg-to-adult viability previously reported (Nepoux et al. 2010), a positive correlation of breeding values here would indicate that the correlation observed in Nepoux et al. 2010 was due to additive genetic effects and not to inbreeding depression.

Does the inbred value predict the breeding value for these traits? In the previous work done on the inbred lines [138], the observed genetic correlation between learning ability and egg-to-adult viability could be due to the effects of a few highly deleterious recessive pleiotropic alleles. In other words, the correlation could be due to inbreeding depression. Such an effect could hide other interactions between genes, and hence the additive effects of the lines. If we fit a regression between the inbred value of the lines (i.e. their genotypic value), and their breeding value, pure additivity would result in a positive slope and an intercept

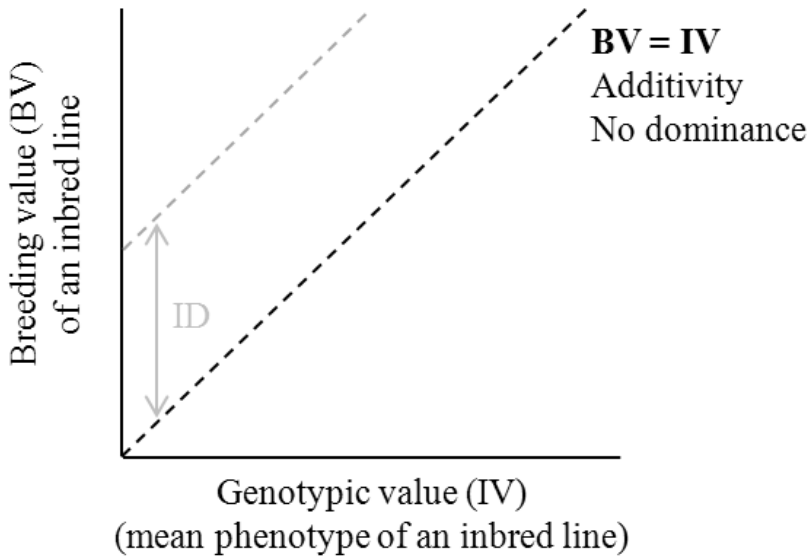

Figure 18: Expected relationship between breeding value and genotypic values of the inbred lines. If the slope of the line is 1, there is pure additivity and absence of dominance, the breeding value would equal the genotypic value (black dashed line), except for the error of measure. Under this condition, an intercept above 0 would indicate inbreeding depression on the trait (grey dashed line). If the slope is smaller than 1, but superior to zero, it still indicates additive effects and an intercept higher than 0 would indicate dominance effects.

equal to 0 (Fig. 18). A positive intercept would represent the dominance deviation. If the slope was close to 1, with a positive intercept, this intercept would represent the inbreeding depression (Fig. 18).

Are the traits we measured affected by inbreeding? It is well established that inbreeding depression impairs many traits. In *Drosophila*, a recent study previously showed inbreeding depression on learning ability and egg-to-adult viability in the same inbred lines as the ones we used [138]. As a related question, we measured the impact of inbreeding on the traits we measured.

The diallel cross, a powerfull tool In this study, we used the inbred lines which have been obtained and characterised for learning ability and development traits by Nepoux and colleagues [138] to address our four questions. We used a diallel cross design, i.e. the crosses of each line with all other lines. We performed a full diallel cross that included all outbred crosses (each line being mated with other lines both as mother and father), and inbred self crosses (crosses of each line by itself). In *Drosophila*, genetic architecture of olfactory discriminative avoidance learning has also been studied with a diallel cross between inbred lines in 1983 by Hewitt and colleagues[76]. They found a low narrow-sense heritability and

a strong dominance deviation by studying crosses of different laboratory populations.

2 Material and methods

2.1 Inbred lines

The inbred lines have been described in [138] (see part I). They were initiated from flies collected in a natural population in Valais (Switzerland) in fall 2007. They have been obtained by sib-matings over 12 generations (inbreeding coefficient $0.75 < F < 0.93$) and then maintained as standard lines (200-300 individuals/line) on normal 8% yeast food [39], with addition of propionic acid as antifungal (6ml for 1L water in the food), under standard laboratory conditions (25°C, 60% relative humidity, 12:12 light-dark cycle). Twelve of these inbred lines were used in this study.

2.2 Diallel cross design

We performed a full diallel cross with all twelve inbred lines using each line both as a mother line and as a father line. It resulted in 144 crosses including 12 self (inbred) crosses. Reciprocal crosses permitted estimation of maternal and paternal effects (see below).

To make crosses, virgin females from each inbred line were collected under CO_2 anaesthesia and isolated in groups of 10 for one week to ensure they were virgin before crossing. Males were collected at the same time. Crosses were performed by pooling 15 virgin females of the mother line and 10 males of the father line. Flies were let mate for two days on fresh fruit jelly with dry yeast. For practical reasons, the full diallel cross was divided into two cross blocks of 72 crosses each (see Fig. 19). For each trait, replicate 1 of cross block 1 was tested on one day, replicate 1 of cross block 2 was tested on the following day and the second replicates of each cross block were tested on the two following days in the same order.

2.3 Traits measurement

2.3.1 Short-term memory

Because of practical limitations (flies did not lay enough eggs for some crosses), we collected up to approximately 100 eggs for each cross. Two days before conditioning, flies were anaesthetised and split into two approximately equal groups which were then maintained on fresh normal food, one group for each conditioning direction. Groups of sexes-mixed flies, aged 5

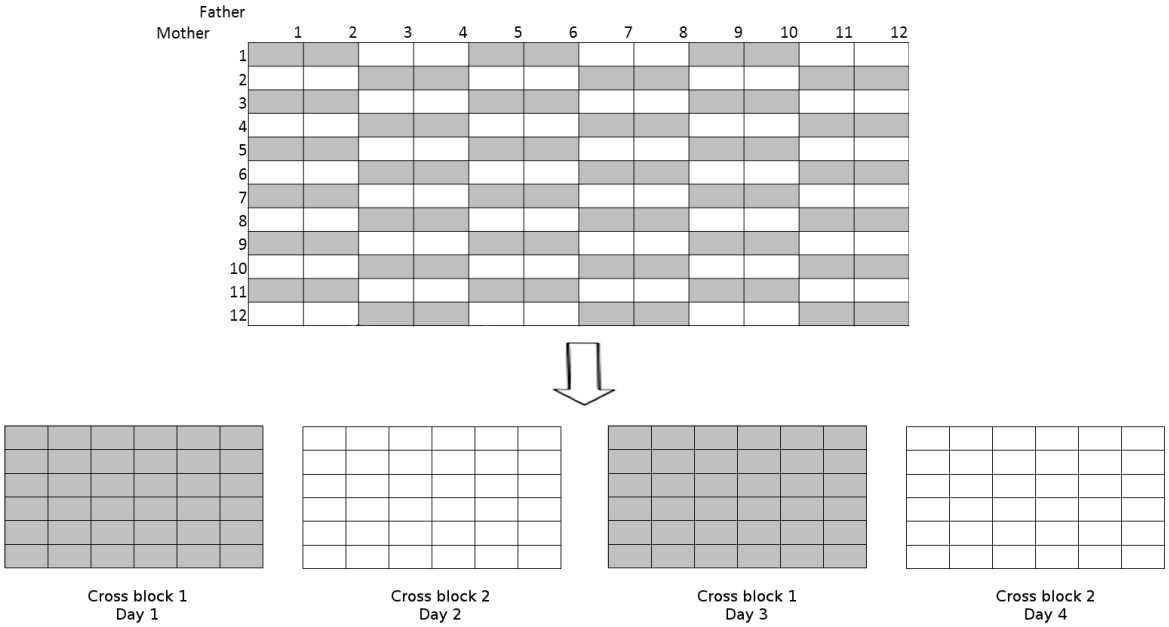

Figure 19: Division of the full diallel cross into two cross blocks of 72 crosses. Grey crosses form cross block 1, and white crosses form cross block 2. They were tested during four consecutive days.

to 7 days, were tested in an aversive olfactory conditioning procedure based on the association of an odorant with mechanical shock [130]. Flies were transferred without anaesthesia to test tubes. During the conditioning phase, three conditioning cycles were applied to flies (it has been previously demonstrated to allow the maximum difference between inbred lines and the outbred base population, [138]). One conditioning cycle consisted of delivering a first odorant for 30s coupled with 1s pulses of mechanical shock, followed by a break with humid air for 60s. Then, a second odorant was delivered for 30s without shock, followed by another break with humid air for 60s to complete the cycle. The odorants used to condition flies were octanol (0.6mL/L paraffin oil) and methylcyclohexanol (MCH, 0.6mL/L paraffin oil). Short-term memory retrieval was tested 2 to 6 minutes after the end of the conditioning phase. Flies were loaded in the central chamber of a T-maze and received the two odorants simultaneously, one odorant in each arm of maze. Flies were allowed to move freely and choose one arm of the maze for 1 minute. Subsequently, flies in each arm of the maze were counted and learning score was calculated as the difference between proportion of flies choosing odorant A when B was reinforced with shock and proportion of flies choosing odorant A when A was reinforced with shock. For this calculation, flies which remained in the central chamber of the maze were excluded. The scale of learning scores range from -1 to 1, zero indicating the absence of associative learning. A negative score would mean that flies learned to like the odorant associated with shock. For each cross within each cross block, two learning scores were calculated based on two groups of flies each, one group trained in

one conditioning direction.

2.3.2 Egg-to-adult viability and developmental time

Egg-to-adult viability and developmental time were measured on a food medium containing only 0.8% of yeast (compared to 8% in the standard food medium used to maintain the lines [39]) to magnify between-cross differences. For each cross, two replicates of 200 eggs were placed in 175mL bottles with 30ml of food, and the emerging adults were counted every day until the duration of the emergence period exceeded the duration of development (number of days from egg collection to adult emergence) to avoid generation overlap. Eggs for one replicate (i.e. for the two cross blocks) were collected on two consecutive days (one block per day) and eggs for the other replicate were collected the two following days and could reveal a maternal effect. As eggs of all lines could not be collected by a single experimenter, lines were randomly assigned to experimenters on each day. The proportion of adult emergence was used as a measure of egg-to-adult viability. Developmental time was measured as the difference in days between the day of egg collection and the day on which emerged flies were counted.

2.3.3 Resistance to infection

We used survival rate after infection as a measure of broad resistance to bacterial infection with a virulent pathogen. Two replicate groups of 30 mated females per cross were collected under CO_2 anaesthesia and let to recover on regular food for at least 24 hours. Bacterial injection was also performed under CO_2 anaesthesia by pricking flies on one side of the thorax with a needle previously dipped into a suspension of the virulent wild-type strain of *Pseudomonas entomophila* [188] at 25% of OD~200 (2.5×10^{10} cells per ml). Additionally, one group of 30 females was pricked with 0.9% saline (buffer used to suspend bacteria at the concentration mentionned above) to control for mortality induced by pricking itself. Survival was then checked every 8 hours for 4 days. As not all flies were dead at the end of the assay, the survival fraction per cross at the last time point of the assay was used as variable for data analysis.

2.4 Data analysis

2.4.1 Nuclear and extra-nuclear contributions in the observed variation

For the variance composition analysis, the data set from each trait included 132 crosses, excluding self crosses statistical correlation between the breeding values of the lines.

We used the Cockerham and Weir's model, which includes nuclear contributions of the mother line i and father line j (n_i and n_j), the interaction between these two particular nuclear contributions (t_{ij}=t_{ji}), extra-nuclear maternal (m_i) and paternal (p_j) effects, and $e_{ijk}$the error [30]. The phenotypic value X_{ijk} of the replicate k of the cross between mother line i and father line j is the following:

$$X_{ijk} = \mu_X + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk} \quad (1)$$

From this model, the total variance of X_{ijk} is the following:

$$\sigma_x^2 = 4\sigma_n^2 + \sigma_t^2 + \sigma_m^2 + \sigma_p^2 + \sigma_e^2 \quad (2)$$

To estimate the different variance components and the breeding values (here equal to twice n, the nuclear additive effects), we implemented the following mixed-effect models that included all these parameters as random effects (nuclear and extranuclear contributions, cross-specific line interaction and error). The model also included fixed effects, which were mean phenotype μ, experimenter (exp), block (day, d) (which was treated as a fixed effect as it had only 4 levels, and contained the cross block effect into it), and time at pricking for resistance to infection data (tp). E_{ijk} corresponded to the residuals, and included the repetition effect (two replicates per cross for each trait) and the final residuals of the models. The L_{ijk} model stands for learning score data, S_{ijk}model for resistance to infection data, V_{ijk}model for egg-to-adult viability data, T_{ijk}model for developmental time data.

$$L_{ijk} = \mu + d + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk}$$

$$S_{ijk} = \mu + d + tp + exp + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk}$$

$$V_{ijk} = \mu + d + exp + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk}$$

$$T_{ijk} = \mu + d + exp + n_i + n_j + t_{ij} + m_i + p_j + e_{ijk}$$

Three out of four traits (learning score, egg-to-adult viability and resistance to infection) are binomial. Because of the distribution of residuals and because the data set is not perfectly balanced, variance components were then calculated using generalized mixed models (GLM) with a binomial distribution (probit link function) for short-term memory, resistance to infec-

tion, and egg-to adult viablity, and a gaussian distribution for developmental time (logarithm link function). The GLM was fitted with extended quasi likelihood method (quasi-REML), calculated with the hglm package [163] of the R software [184]. Learning score data were transformed to be treated as proportions instead of scores in the GLM analysis (transformation (stm+1)/2) and multiplied by the total number of flies tested to account for differences between groups of flies tested.

For each traits, we obtained the effects and their corresponding variance components on the transformed scale (probit scale for short-term memory, resistance to infection and egg-to-adult viability; log scale for developmental time).

The significance of the effects has been calculated with model comparison on the base of cAIC.

2.4.2 Additive genetic correlations between traits

Correlations were calculated for each pair of traits by extracting the estimates of additive nuclear effects calculated without self crosses from the GLM models. Significance of the correlations was tested with a Pearson's correlation test. Because values for one trait were used more than once, we applied a Bonferoni correction for multiple tests (n=6), which lowered the threshold for test significance to 0.05/6=0.008. Note that additive nuclear effects used for the correlations were calculated on the transformed scale.

2.4.3 Correlations between breeding values and inbred (genotypic) values

The correlation has been calculated with the mean genotypic values of self crosses and the estimations of additive nuclear effects extracted from the GLM analysis described above (which excluded self crosses from the data sets). Two replicates per case of the diallel cross were set. Consequently, the estimation of the breeding values, which rely on all crosses for a line (44 values per line), was more precise than the calculation of the inbred values, which relies on only two replicates. Note here again that additive nuclear effects were obtained on the transformed scale.

2.4.4 Effect of inbreeding

The same models described above were fitted on the complete data sets including 132 outbred crosses and 12 inbred crosses for each trait. A fixed inbreeding effect was added to the model to test for inbreeding depression on the traits we measured. The estimates for the effect were calculated on the transformed scale.

Table 3: Partitioning of the variance of random effects (estimates [95% CI] on the transformed scale, except σ^2_e) intof nuclear genetic variance (half breeding values, σ^2_n), interaction of nuclear contributions variance (σ^2_t), paternal extra-nuclear variance (σ^2_p) and maternal extra-nuclear variance (σ^2_m) and residual variance (σ^2_e) for learning score ((stm+1)/2), resistance to infection, developmental time and egg-to-adult viability. Variance components were calculated with all heterozygous crosses of the full diallel table excluding all self crosses between inbred lines.

Source of variation	Learning score	Resistance to infection	Developmental time	Egg-to-adult viability
σ^2_n	0.0121053 [0.005 – 0.03]	0.0843574 [0.04 – 0.2]	0.0003508 [0.0001 – 0.0009]	0.0003780 [0.00001 – 0.01]
σ^2_t	0.0006441 [0.00009 – 0.005]	0.0077577 [0.002 – 0.03]	0.0003446 [0.0002 – 0.0006]	0.0072901 [0.003 – 0.02]
σ^2_p	0.0000093 [$7^{e-15} – 1^{e+4}$]	0.0000168 [$2^{e-18} – 1^{e+8}$]	0.0000033 [$4^{e-10} – 0.03$]	0.0015703 [0.0002 – 0.01]
σ^2_m	0.0001198 [$4^{e-7} – 0.04$]	0.0000172 [$5^{e-18} – 8^{e+7}$]	0.0020741 [0.0009 – 0.005]	0.2240134 [0.1 – 0.5]
σ^2_e	0.0352192	0.0666307	0.4852852	0.0844999

3 Results

3.1 Nuclear and extra-nuclear contributions in the observed variance

The model comparison using cAIC showed that the nuclear additive contribution was the only significant effect for learning ability. Concerning resistance to infection, nuclear additive contribution and interaction of nuclear contribution were significant. In developmental time, nuclear additive contribution, interaction of nuclear contribution and maternal effects were significant. Finally, in egg-to-adult viability, only maternal effects contributed significantly to the total variance.

In the total variance, nuclear genetic variance σ^2_n was higher than interaction of nuclear contributions variance σ^2_t for all traits except egg-to-adult viability (Table. 3). The ratio σ^2_n / σ^2_t varied among traits from $\sim$1 for developmental time, to $\sim$10 for resistance to infection and $\sim$19 for learning score. This ratio was 0.05 for egg-to-adult viability meaning that σ^2_t was $\sim$20 times higher that σ^2_n for this trait. Fig. 21 shows the n, t, m and p effects as deviations from zero, on the transformed scale, and Fig. 22 shows the variance of the effects, back-transformed on the original scale.

The figure 20 shows whether each inbred line contributed equally to the phenotype when used as mother and as father line in the crosses. It reveals that for egg-to-adult viability

and developmental time, the inbred lines contributed to the phenotype mostly when used as mother lines, suggesting that the maternal extra-nuclear effect may be larger than the other effects for these two traits (Fig. 20). A similar but less strong difference in line contributions as father and mother appeared for learning score, while nearly no difference was observable for resistance to infection.

These observations were confirmed by the results of the GLM analysis. The ratio σ_m^2 / σ_p^2 was $\sim$140 for egg-to-adult viability and $\sim$630 for developmental time. Actually, paternal extra-nuclear variance was not significant. Maternal extra-nuclear variance explained a much larger amount of the total variance than nuclear genetic variance and the variance of interaction of nuclear contributions (Tab. 3). The ratio σ_m^2 / σ_p^2 was smaller for learning score ($\sim$13) suggesting that later in a fly life, maternal effects become smaller, but still maternal extra-nuclear variance was larger than paternal extra-nuclear variance. However, for this trait, maternal variance was very small compared to nuclear genetic variance σ_n^2 (Tab. 3). As an exception, resistance to infection showed similar small amounts of maternal and paternal extra-nuclear variances in the total variance, which were both very small relatively to nuclear variances as for learning score. As expected from the results, the sum of extra-nuclear variances (maternal and paternal effects) was at least 10 times smaller that the sum of nuclear genetic variances ($\sigma_n^2 + \sigma_t^2$), except for development traits.

Residual variance was either of similar size as nuclear variance (short-term memory, resistance to infection) or more than 200 times higher (egg-to-adult viability, developmental time). Extra-nuclear maternal variances were globally very small compared to residual variance.

Several fixed effects were included in the GLM models to test for block, experimenter effects on the total variance and more importantly to remove these parts of the total variance for the calculation of estimates of the different variance components. Except for learning score and resistance to infection, a part of the variance was accounted for by variation among blocks. For development traits, there was no big effect of experimenter. On the opposite, experimenter had a strong effect on resistance to infection ($p<0.001$), presumably because of speed differences at pricking. The time at which pricking was performed did not affect the fly's resistance afterwards.

3.2 Additive genetic correlations between traits

None of the correlations between the additive nuclear effects of traits were significant (fig. 23). Nevertheless, the correlation between developmental time and egg-to-adult viability tended to be negative before Bonferoni correction for multiple tests (r=-0.6; p=0.03). Nuclear additive effects were very small, probably inexistant, in developmental traits. If we calculate the correlation between GCA values, which include the parental extra-nuclear effects, none of them was significant either. Note that the coefficient of correlation between resistance to

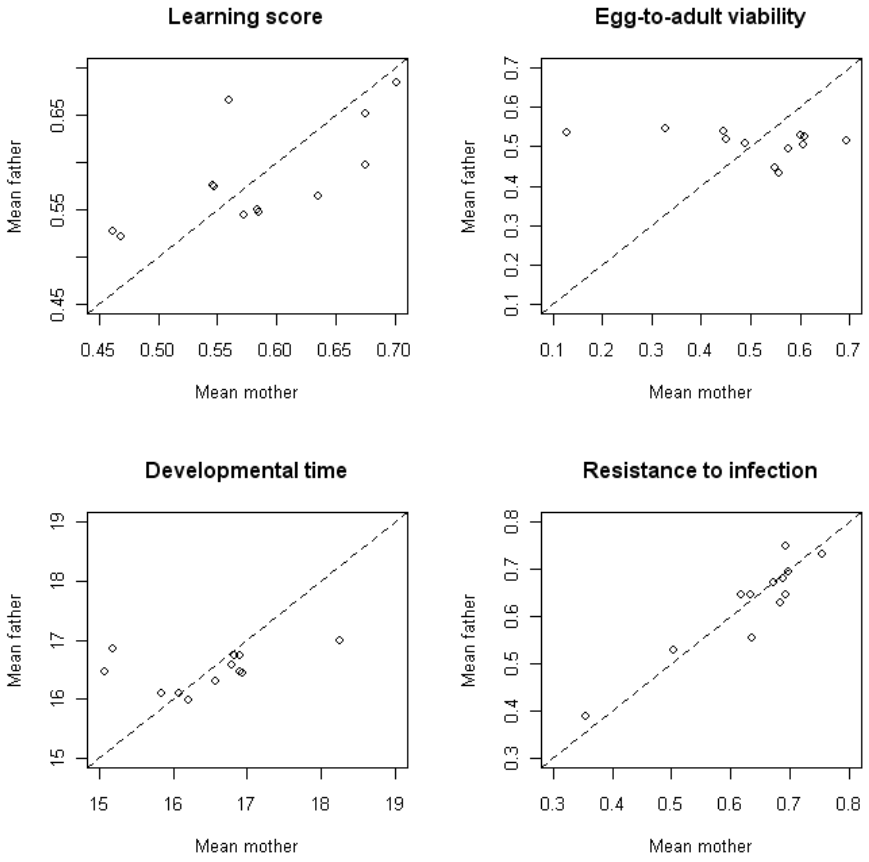

Figure 20: Mean phenotype of the inbred lines when used as father lines against mean phenotype of the same lines when used as mother lines for each trait we measured. Inbred crosses have been removed. The dashed line illustrates equal contributions to the phenotype of one line when used as father and mother line.

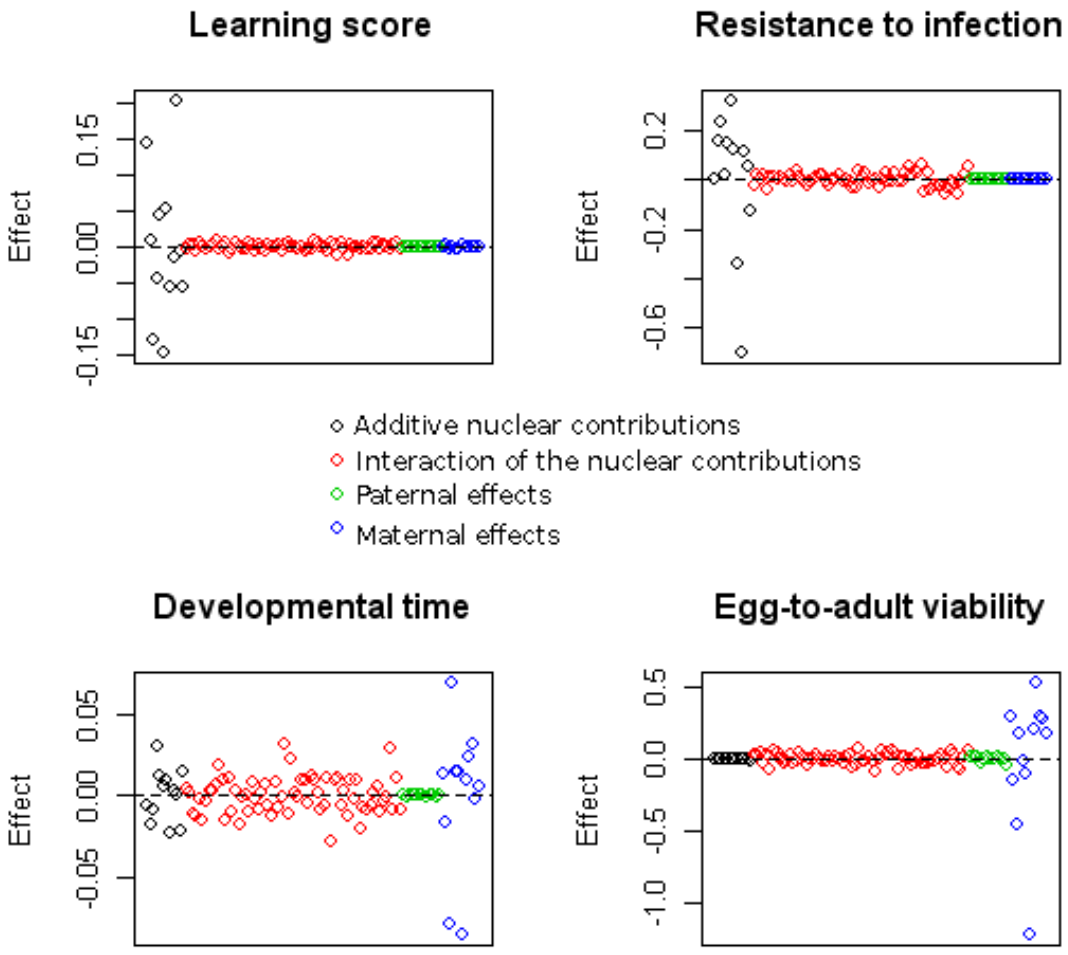

Figure 21: Estimates for additive nuclear contributions, interaction of the nuclear contributions, extra-nuclear paternal and extra-nuclear maternal effects, for each traits we measured, per inbred line and crosses . The dispersion of the points reflects the importance of the effect.

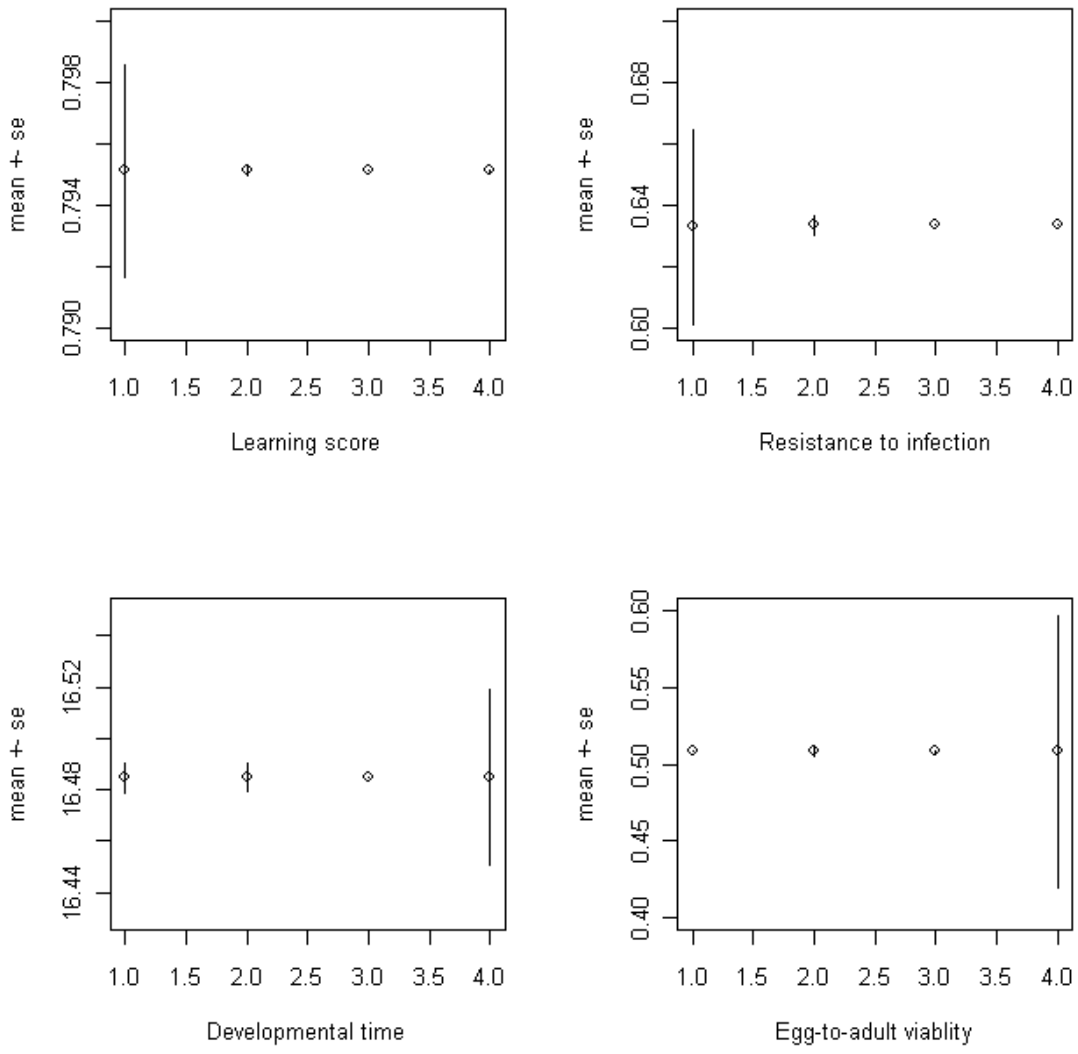

Figure 22: Back-transformed variances for nuclear and extra-nuclear effects, relative to mean of the traits (mean±s.e.).

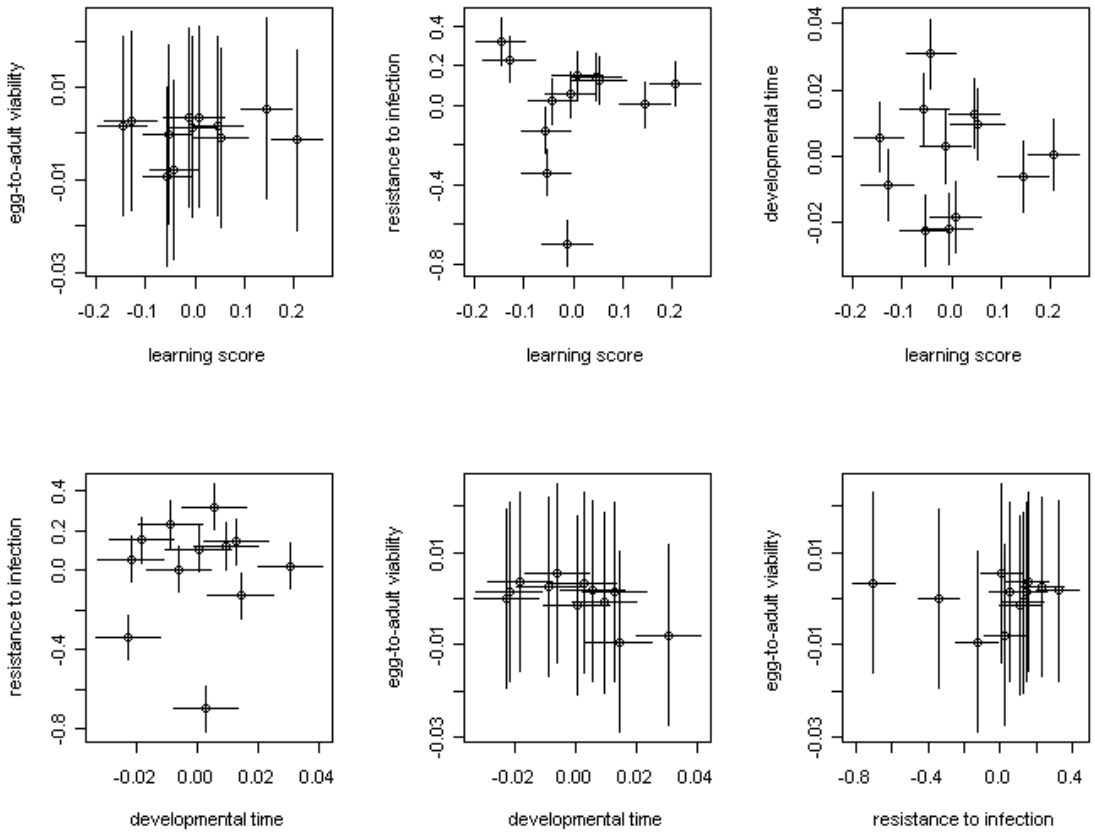

Figure 23: Additive genetic correlation between traits (correlations between additive nuclear effects) ±s.e..

infection and viability was high, even not significantly negative (r=-0.49; p=0.1). All the results are summarized in Tab. 4.

3.3 Correlations between breeding values and inbred values

The correlation between the breeding values and the inbred values was significantly positive for resistance to infection (r=0.9, p=0.0004; linear regression: y=-0.5 + 1.04x). This correlation was mostly driven by two extreme lines with very low breeding values (Fig. 24). It tended to be significantly positive for learning score (r=0.66, p=0.04; linear regression: y=-0.35 + 0.64x), but were not significant for developmental time (r=0.42, p=0.20; linear regression: y=-0.059 + 0.0034x) and egg-to-adult viability (r=0.16, p=0.6; linear regression: y=-0.0011 + 0.0028x). These results are presented in Fig. 24.

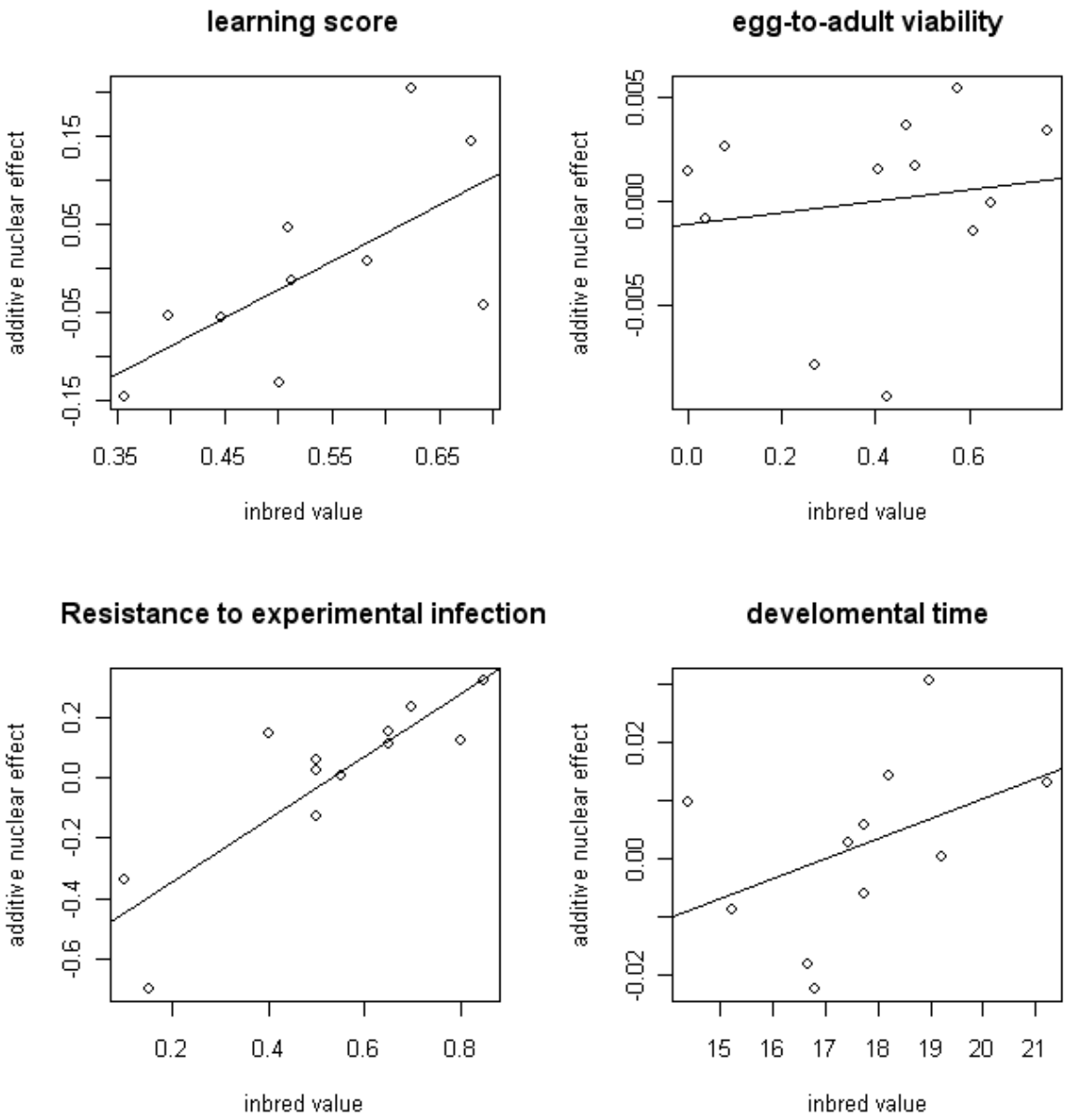

Figure 24: Correlation between inbred values and nuclear genetic effects for each trait.

Table 4: Table of correlations between additive effects. The right side of the diagonal represents the correlations between additive nuclear effects only (n), the left side correlations between GCA (GCA=2n+p+m).

	Learning score	Resistance to infection	Developmental time	Egg-to-adult viability
Learning score	-	r=-0.02 p=0.95	r=-0.02 p=0.96	r=0.15 p=0.64
Resistance to infection	r=-0.02 p=0.95	-	r=-0.07 p=0.83	r=0.03 p=0.93
Developmental time	r=0.10 p=0.75	r=-0.15 p=0.63	-	r=-0.6 p=0.03
Egg-to-adult viability	r=0.18 p=0.58	r=-0.49 p=0.10	r=0.31 p=0.33	-

3.4 Effect of inbreeding

First of all, by constrast with what was previously reported [138], inbreeding depression did not affect significantly learning score (mean effect estimate $\pm$ s.e.: -0.11 $\pm$ 0.06, p=0.08; effect of inbreeding on mean phenotype of inbred crosses relatively to outbred crosses: -23%) nor egg-to-adult viability (-0.18 $\pm$ 0.14, p=0.19; -25%). However, developmental time was affected by inbreeding (0.06 $\pm$ 0.02, p<0.0001; -7%). Inbreeding also significantly impaired resistance to infection (-0.33 $\pm$ 0.12, p=0.005; -24%).

4 Discussion

Nuclear and extra-nuclear contributions in the observed variance

The nuclear genetic variance σ^2_n was higher than the variance of interaction between nuclear contributions σ^2_t for all traits except egg-to-adult viability. In our study, the variance σ^2_n corresponds to the variance of half the breeding values, and represents the additive variance of the nuclear parental contributions. This variance component also includes variance of epistatic effects, which we can be measured on the F2 progeny of the diallel cross, and which we ignored here [199]. The variance of interaction of nuclear contributions σ^2_t is a cross-specific variance, and represents the interaction between the maternal and paternal nuclear contributions. High ratios of σ^2_n/σ^2_t suggests that, for the traits we measured, the genotypic value of the progeny may less likely deviate from the mean genotype of the parental lines and hence the progeny may more likely exhibit intermediate genotypic values. For egg-to-adult viability, both σ^2_t and σ^2_n are very small, almost nonexistant. Nevertheless, higher σ^2_t than σ^2_n

suggests that the phenotype of the progeny may depends more on the interactions between alleles of the parents than on the additive effects of these alleles *per se*.

As far as reciprocal effects are concerned, we found that extra-nuclear maternal variance was large relatively to extra-nuclear paternal variance for all traits except for resistance to infection, for which they were both extremely and equally small (this indicates that extra-nuclear efffects may not contribute a lot to the ability to resist bacterial infection of the progeny of parental lines crosses). These are expected results when considering the uniparental (maternal) heredity of the mitochondrial DNA plus the cytoplasm which is part of the environment for gene expression. Different female flies likely invest different amounts of nutrients when maturing eggs, leading to differences in development traits. Moreover, for the development traits, the extra-nuclear maternal variance is higher than nuclear additive variance, which is close to zero. This suggests that the estimation of total additive variance of a line should include part of maternal variance because additive effects due to mitochondrial DNA and epigenetic components cannot be neglected.

The extra-nuclear maternal variances were much smaller than nuclear additive variances for traits measured at the adult stage, indicating that for learning ability and resistance to infection, σ_n^2 is a good estimator of the total additive variance of the lines. Hence, phenotypes of the progeny may be more under the control of the nuclear genetic contributions of their parents than under the control of non-nuclear maternal effects for these traits. This suggests that the maternal effects are mostly important in developmental stage, and that their role fades at adult stage. Nevertheless, a recent study by Burns and Mery [24] showed an effect of the mother age on the fly progeny performance in the same conditioning procedure. This suggests that extra-nuclear maternal effects can have a significant effect on learning ability even at the adult stage.

Additive genetic correlations between traits

All the observed correlations were non-significant except the correlation between developmental time and egg-to-adult viability. In the previous work mentionned above [138], the correlation between the learning ability and the egg-to-adult viability of the same inbred lines was significantly positive. The additive genetic correlation between these traits is here not significant probably because σ_n^2 is very small for the development traits and additive genetic variance was included in the extra-nuclear maternal variance σ_m^2 (although including σ_m^2 into breeding values does not make the correlations significant). The absence of significant additive genetic correlation the two traits does not mean the absence of pleiotropy, because it is possible that the traits are under the control of two genes or pools of genes acting with the same amplitude but in opposite directions (one increasing one trait while decreasing the other trait, the other acting in the opposite direction, or one increasing both traits and the other

acting in the opposite direction). An absence of correlation may also mean that the traits are not genetically related, and hence evolve independently. When considering the results of the previous study [138], it also indicates that the correlation previously observed between these two traits was mostly due to inbreeding effects, which might have masked weak genetic relationships.

There was no additive genetic correlation between learning ability and resistance to infection. This is consistent with the results reported in previous studies in *Drosophila* [97] and a social insect [6]. As mentionned above, this does not mean that pleiotropic effects are absent. However, if this turned out that the two traits are under the control of different pools of genes evolving independently, our results combined with the learning impairment upon immune-challenge observed in social insects [115, 158] would suggest that this negative relationship may occur only because of resource allocation trade-offs. The correlation between resistance to infection and egg-to-adult viability tended to be positive when calculated from GCA estimates. The correlation between the maternal effects of the two traits was not significant ($p=0.77$), so it suggests that the nuclear genes can only be expressed at their highest level when the maternal effects are favourable, contradicting hypothesis of pleiotropy.

A negative trend for additive genetic correlation was observed between developmental time and egg-to-adult viability (which was not observable between GCA). This means that the flies with the highest egg-to-adult viablity exhibited the fastest development. As fitness is probably high for both high egg-to-adult viability and quick developmental time, this suggests that the relationship between these traits may be due to non-antagonistic pleiotropy. Alternatively, our outbred base population was large (more than 1000 individuals/generation) but as any real population with possibly sexual selection occurring, it cannot be expected to fulfill the panmixia condition. Therefore, there might be some linkage disequilibrium, which can also cause a correlation between traits. Another possible explanation comes from the fact that both egg-to-adult viability and developmental time were measured on the same flies reared in the same vial. The correlation between the two traits thus includes covariance of vial effects.

Correlations between breeding values and inbred values

The correlation between breeding values and inbred values tended to be positive for learning ability. It suggests that the inbred values can predict partially the breeding values for this trait. Neither the slope nor the intercept of the linear regression were significantly different from 0, indicating that the trait may be determined by small additive effects and probably very small dominance effects. This is consistent with the results of the variance partitioning.

The correlation was significant for resistance to infection. The slope of the regression was close to 1, meaning the breeding values were almost equal to inbred values. This suggests that

there is nearly no dominance but mostly additive effects for the trait. This is supported by the results of the variance partitioning that showed high nuclear variance σ_n^2, relatively lower nuclear interaction variance σ_t^2 but very low extra-nuclear maternal and paternal variances. Because the slope was $\sim$1, the value for the intercept gives information about the effect of inbreeding on the trait: if the intercept is positive, there is inbreeding depression while if it is negative, it suggests outbreeding depression. The intercept of the regression for resistance to infection was below 0, indicting inbreeding depression on this trait. However the correlation was mostly due to two extreme lines with very low inbred values and breeding values. When removed from the table, the correlation was still significantly positive but the intercept was not different from 0 (r=0.66, p=0.04; linear regression: slope=0.6, intercept=-0.2).

By contrast, the correlation was not significant for egg-to-adult viability and developmental time. In fact, for these traits, nuclear and nuclear interaction variances were small while extra-nuclear maternal variance explained a large amount of the observed variance. This is supported by the fact that there was a significant positive correlation between inbred values and extra-nuclear maternal variance (linear regression: egg-to-adult viability, y=0.08 + 1.8x, r=0.8, p=0.002; developmental time: y=-0.02+ 0.02x, r=0.77, p=0.005).

Inbreeding depression on learning ability and egg-to-adult viability

We observed a trend for inbreeding depression on short-term memory but no significant effect on egg-to-adult viability. This is contradictory with what was previously reported on the effect of inbreeding on learning ability and egg-to-adult which reported inbreeding depression effect on both traits [138]. It indicates that learning ability was rather stable among studies and not very sensitive to inbreeding depression This confirms results from parts I and II, suggesting that learning ability is under high selection in nature. Nevertheless, the estimate for the inbreeding effect on egg-to-adult viability was quite low and not far from being significantly different from zero (with n=2 replicates per cross), which means that the test was not powerfull enough. Outbred crosses exhibited higher phenotypic values (heterosis) than inbred crosses for both short-term memory (+23%) and egg-to-adult viability (+ 25%).

Developmental time was significantly higher in outbred crosses (7% longer), meaning that inbred crosses took more time to develop and flies to emerge. This is consistent with the presence of recessive deleterious alleles for this trait in inbred lines. It also further suggests that developmental time and egg-to-adult viability might be affected by partially distinct pools of genes as the effects of inbreeding differed in amplitude.

Similarly, resistance to infection was also higher in outbred crosses than in inbred crosses (24% higher). This is consistent with has been generally reported in the literature on the effects of heterozygosity on resistance to infection (e.g. for MHC in vertebrates, reviewed in

[193]). It also reveals the presence of recessive deleterious alleles for this trait in the inbred lines.

Conclusion

The variance among crosses was mostly explained by maternal effects for the development traits and by nuclear additive effects for learning ability and resistance to infection, measured at the adult stage (a few days after emergence). These results have to be taken with caution because epistatic effects could not be estimated, which could be done with an analysis of F2 crosses.

The positive correlation previously observed between learning ability and egg-to-adult viability of inbred lines is here not significant when the breeding values are used instead of the genotypic values. Knowing that the variance of egg-to-adult viability was mostly due to maternal effects, this may indicate that the learning ability is expressed at a high level when the maternal effects are favourable. No other additive genetic correlation was significant between the traits we measured. This can mean that the traits are not genetically related, but we cannot eliminate the hypothesis of pleiotropy.

Inbred values were a good predictor of the breeding values fo the lines for the traits measured at adult stage, but not for development traits which were mostly determined by maternal effects.

Finally, our study allowed us to calculate the effects of inbreeding depression on the four traits we measured, even if the measurements of inbred lines and outbred crosses are highly unbalanced in terms of numbers of values, which means that these results have to be interpreted with caution. We found no significant inbreeding depression for learning ability nor egg-to-adult viability, but a significant inbreeding depression for developmental time and resistance to infection. This could be due to recessive deleterious alleles homozygous in the inbred lines.

Part V

General discussion and conclusion

Learning ability is a quantitative and complex trait that is expressed in many different behavioral contexts (foraging, predator and pathogens avoidance, mating...), levels (from simple forms of learning like habituation to complex forms of learning relying on association or imitation) and modalities (learning can be based on different sensory cues, that rely on different neuronal pathways). It is widespread in the animal kingdom and is present even in some unicellular organisms [197, 91]. Learning plays a central role in animal life and evolution, although it is a very costly ability, especially in terms of energy for neuronal synthesis and memory formation [103]. It has also been shown in *Drosophila* that learning ability is constrained by evolutionary trade-offs with other fitness related traits like lifespan [23] or larval competitive ability [129, 96]. This suggests antagonistic pleiotropy, which under certain conditions may favor genetic variability for learning because it allows the emergence of several strategies that can respond to various environmental pressures and therefore respond to balancing selection [160, 164, 75]. Consequently, to understand how learning ability evolves under such selection pressures and genetic interactions, it is necessary to investigate its genetic variability in natural populations. This allows one to ask several questions: what is the amount of genetic variation for learning in natural populations? What is the part of additive variance, is there maternal effects? Are there genetic correlations between learning and other traits related to fitness?

In order to answer these questions, I studied the quantitative genetics of learning in a natural population of *Drosophila melanogaster* in Valais (Switzerland). I used approaches to investigate the variation in the population's learning ability: inbred lines and isofemale lines. Firstly, I collected an outbred base population and derived multiple lines inbred during 12 generations. Theses inbred lines, almost homozygous, theoretically represent a sample of random haplotypes in the population, if we assume no selection pressures acting during the inbreeding process. During this process, some inbreeding depression may appear, causing a decreases of the mean of a trait in inbred lines compared to the outbred base population. Three mechanisms may cause inbreeding depression: overdominance, dominance and epistasis. This last mechanism is most difficult to identify and is commonly neglected.

Does inbreeding affect learning ability in *Drosophila*? I compared the outbred base population to the mixed inbred lines in order to measure the inbreeding depression in learning ability (associative short-term memory). A fitness-related trait was also measured: egg-to-adult viability. Nevertheless, inbreeding depression decreased learning ability, especially memory acquisition (up to 20% for 3 cycles of conditioning), only after several generations of inbreed-

ing, but this effect was mild compared to egg-to-adult viability. Moreover, even in the highly inbred lines, every line showed some learning ability after 12 generations of inbreeding. This suggested that alleles impairing learning ability are eliminated by selection, and therefore that learning ability is under strong selection in natural populations of *Drosophila*. It also suggests that alleles reducing learning performance are on average partially recessive, because their effect does not appear in the outbred base population. Moreover, overdominance seems unlikely as major cause of the inbreeding depression, because even if the overall mean of the inbred line is smaller as the outbred base population, some of the inbred lines show the same learning score as the outbred base population. If overdominance played an important part in inbreeding depression, then all the homozygous lines should show lower learning ability than outbred base population.

Is inbreeding depression for learning due to the same genes as the inbreeding depression for egg-to-adult viability? The positive correlation I observed between egg-to-adult viability and memory contrasts with the trade-off found in other studies [129, 96, 23]. This correlation indicates that the genes responsible for loss of learning ability could be pleiotropic genes involved in fitness-related functions other than learning ability. This observation cannot explain the maintenance of genetic variation, as directional selection should act to eliminate alleles that are deleterious for several traits. Nevertheless, a small number of recessive pleiotropic, generally deleterious alleles may hide other genetic interactions.

Are deleterious alleles purged from the inbred lines during the inbreeding process? The loss of many inbred lines during the inbreeding process showed that selection for viability and fecundity acted strongly, although we could not find evidence for purging of deleterious alleles. The cross of all the inbred lines did not result in a population that performed better than the outbred base population.

To investigate the positive relationship between learning ability and egg-to-adult viability, I decided to use two complementary methods. First, to perform a new sampling in the same natural population and to derive isofemale lines, in order to obtain a new estimates of the genetic variance and genetic correlations in lines that may have not fixed rare deleterious recessive alleles and may be a better sampling of the natural population. Second, to cross of all the inbred lines in order to partition the variance into its genetic and non-genetic components and therefore analyze the amount of additive genetic variance in the population, and estimate the additive genetic correlations between the traits.

During the inbreeding process of the inbred lines, the genetic variance was redistributed: the within-line variance decreases whereas the between line variance increases. This was due to the allele fixation: intermediate genotypes, and therefore heterozygosity between additive alleles, were lost. In isofemale lines, the inbreeding coefficient is much smaller ($F=0.25$; [77]) because the lines are initiated from four haplotypes and there is still within-line genetic

variance. In absence of selection during the inbreeding process, the among-line variance of the inbred lines is expected to be larger than the among-line variance of the isofemale lines. Nevertheless, even if we could not find evidence of purging, we cannot eliminate this hypothesis. Linkage disequilibrium may also have caused the loss of alleles not related to these functions, through genetic drift. In isofemale lines, the allelic diversity should have been conserved better and be closer to the allelic diversity of the natural population, and inbreeding depression should be less important.

The observed variance amount the isofemale lines was significant and higher to the variance between inbred lines, even it is hazardous to compare the results from two different experiments. This confirms that some alleles have been lost during the inbreeding process. Moreover, the correlation between learning ability and egg-to adult viability (nor developmental time) was not significant in the isofemale line experiment. This may indicate that the inbreeding depression for these two traits in the precedent experiment was indeed hiding other genetic interactions. The hypothesis of pleiotropy cannot be eliminated: two genes or pools of genes may act in opposite direction. I also tested for correlations between learning ability and two other fitness-related traits: developmental time, which is a developmental trait like egg-to-adult viability, and resistance to infection. This last trait was measured because a link between learning ability and immunity has been suggested from studies in social insects [115, 158, 5, 6]. No significant relationship between learning ability and developmental time nor resistance to infection was shown. This last result is consistent with prior work on the relationship between learning ability and parasitoïd resistance in *Drosophila* [97].

In order to investigate the among of additive variance in total genetic variance of the inbred lines, and to analyze the additive genetic correlation between learning ability and other fitness-related traits that we previously measured, a diallel cross was performed. The twelve remaining inbred lines have been crossed with each other in order to measure the performance of each cross in four traits: learning ability, resistance experimental infection, egg-to-adult viability and developmental time. This allowed me to partition the total variance into several components (Cockerham&Weir model [30]): first, the additive effects of nuclear genes; second, the interaction between nuclear effects in a cross, which indicates its dominance deviation, i.e. the deviation between its value and a value expected from the nuclear effects of the parental lines; and finally, the maternal and paternal extra-nuclear effects. The results have to be interpret with caution, because epistatic interaction between loci cannot be eliminated, and maternal effects carry also additive genetic components corresponding to mitochondrial DNA and epigenetic effects. Nevertheless, the variance partitioning revealed that for learning ability and resistance to experimental bacterial infection, the variance of the nuclear additive effects was larger than the other variance components. On the opposite, maternal effect variance was larger than other variance components for the development traits

like developmental time and egg-to-adult viability. This may indicate that maternal effects are important during development and that their effect fade in adult flies. Nevertheless, it has been previously shown in *Drosophila* that maternal effects can also influence the learning ability of adult flies (old mothers's progeny showed decrease in learning ability compared to young mother's progeny) [24].

Concerning the correlation between learning ability and egg-to-adult viability, we could not identify any significant relationship. Nevertheless, we cannot distinguish between two hypothesis: pleiotropy between the two traits (two genes or pools of genes acting the first to increase the traits in the same time, the second to increase one trait while decreasing the other), and independence of the two traits. As the variance of viability was mostly due to maternal effects, this suggests that high learning ability can only be expressed if the maternel effects are favourable for developmental traits. No significant correlation was found between learning ability and other fitness-related traits like developmental time or resistance to experimental infection, which also suggests that either the traits are under the control of independent pools of genes or they are under the control of two pleiotropic pools of genes acting in different direction. Nevertheless, even if it was not statistically significant, a positive trend was observed between the GCA (GCA=additive nuclear effects+parental effects) of resistance to infection and egg-to-adult viability. As no correlation has been found in maternal effect, it may suggest interaction between nuclear and extra-nuclear effects.

Moreover, the correlation between inbreeding value and breeding value was significant for resistance to infection, but not significant for learning ability, egg-to-adult viability and resistance to infection and does not allow us to detect inbreeding depression. This also indicates that the inbred values do not predict the breeding values for developmental traits nor learning ability.

Finally, the study of diallel cross showed a significant effect of inbreeding depression (7% decrease of the mean of inbred lines compared to the mean of outbred crosses) on developmental time, but no significant effect of inbreeding on learning ability (23% decrease of the mean of inbred lines compared to the mean of outbred crosses) nor egg-to-adult viability (25% decrease of the mean of inbred lines compared to the mean of outbred crosses), which contradicts the first study on inbred lines. Not surprisingly, inbreeding also decrease resistance to infection (24%), which is consistant with expectation from studies on MHC in mammals, which indicate that heterozygous individuals have a higher resistance to infection than homozygous [193]. Nevertheless, these results have to be interpret with caution because the inbred lines were only measured twice, although they have been crosses 22 times.

If we consider the results from these complementary approaches together, they contradict the results of other studies which concluded trade-offs between learning ability and other fitness-related traits, like lifespan or larval competitive ability, which could, under some conditions,

favor maintenance of variation for learning ability. My results, on the contrary, cannot alone explain the maintenance of the genetic variation in learning ability because even if we cannot eliminate the hypothesis of pleiotropy, there is no evidence for any trade-off between the traits. This indicates that, in the case of an environment with a stable rate of change, and therefore no variation in selection for learning ability, selection pressures for the traits we measured much likely favor an erosion of learning ability variation, because no selection pressure would act for a diminishing learning ability.

In order to continue this study, a study of F2 crosses between inbred lines could help to understand the role of epistasis, and therefore estimate the amount of additive and dominance variation in inbred lines more precisely. It could also be interesting to study another wild population, from which more inbred lines have been derived. The lines derived from the population of Raleight (USA) have been entirely sequenced in Trudy Mackay's laboratory and could provide a good material in order to investigate the variance for learning ability [114]. Moreover, once the variation among lines would have been characterized, a QTL detection could be performed in order to locate the loci responsible for this natural variation.

References

[1] S. Abu-Rabia and L. Maroun. The effect of consanguineous marriage on reading disability in the arab community. *Dyslexia*, 11(1):1–21, 2005.

[2] K. Acevedo-Whitehouse, F. Gulland, D. Greig, and W. Amos. Inbreeding: Disease susceptibility in california sea lions. *Nature*, 422(6927):35, 2003.

[3] M. Afzal. Consequences of consanguinity on cognitive behavior. *Behav Genet*, 18(5):583–94, 1988.

[4] O. Ala-Honkola, A. Uddstrom, B. D. Pauli, and K. Lindstrom. Strong inbreeding depression in male mating behaviour in a poeciliid fish. *J Evol Biol*, 22(7):1396–406, 2009.

[5] A Alghamdi, L Dalton, A Phillis, E Rosato, and E B Mallon. Immune response impairs learning in free-flying bumble-bees. *Biol Lett*, 4(5):479–81, Oct 2008.

[6] A Alghamdi, N E Raine, E Rosato, and E B Mallon. No evidence for an evolutionary trade-off between learning and immunity in a social insect. *Biol Lett*, 5(1):55–7, Feb 2009.

[7] Gunjan H Arya, Allison L Weber, Ping Wang, Michael M Magwire, Yazmin L Serrano Negron, Trudy F C Mackay, and Robert R H Anholt. Natural variation, functional pleiotropy and transcriptional contexts of odorant binding protein genes in *Drosophila melanogaster*. *Genetics*, 186(4):1475–85, Dec 2010.

[8] J. Aspi. Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*. *Heredity*, 84 (Pt 3):273–82, 2000.

[9] J. F. Ayroles, M. A. Carbone, E. A. Stone, K. W. Jordan, R. F. Lyman, M. M. Magwire, S. M. Rollmann, L. H. Duncan, F. Lawrence, R. R. Anholt, and T. F. Mackay. Systems genetics of complex traits in *Drosophila melanogaster*. *Nat Genet*, 41(3):299–307, 2009.

[10] P. I. Bader, S. Dougherty, N. Cangany, G. Raymond, and C. E. Jackson. Infantile refsum disease in four amish sibs. *Am J Med Genet*, 90(2):110–4, 2000.

[11] J. D. Ballou. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *J Hered*, 88(3):169–78, 1997.

[12] J. Bashi. Effects of inbreeding on cognitive performance. *Nature*, 266(5601):440–2, 1977.

[13] S C Bernstein, L H Throckmorton, and J L Hubby. Still more genetic variability in natural populations. *Proc Natl Acad Sci U S A*, 70(12):3928–31, Dec 1973.

[14] C. Biemont. Interactions between ageing and inbreeding effects on development of *Drosophila melanogaster* embryos. *Mech Ageing Dev*, 5(5):315–24, 1976.

[15] C. Biemont. Inbreeding effects: evidence for a genetic system which regulates viability in *Drosophila melanogaster* populations. *Mech Ageing Dev*, 8(1):21–42, 1978.

[16] James A Birchler, Donald L Auger, and Nicole C Riddle. In search of the molecular basis of heterosis. *Plant Cell*, 15(10):2236–9, Oct 2003.

[17] W. U. Blanckenhorn. The evolution of body size: what keeps organisms small? *Q Rev Biol*, 75(4):385–407, 2000.

[18] M Boots and M Begon. Trade-offs with resistance to a granulosis-virus in the indian meal moth, examined by a laboratory evolution experiment. *Functional Ecology*, 7:528–534, 1993.

[19] C. Brandes. Estimation of heritability of learning behavior in honeybees (*Apis mellifera capensis*). *Behav Genet*, 18(1):119–32, 1988.

[20] C. Brandes. Genetic differences in learning behavior in honeybees (*Apis mellifera capensis*). *Behav Genet*, 21(3):271–94, 1991.

[21] B. Brembs and M. Heisenberg. The operant and the classical in conditioned orientation of *Drosophila melanogaster* at the flight simulator. *Learn Mem*, 7(2):104–15, 2000.

[22] C J Brown and O D Murphee. The effect of inbreeding on human aversion in pointer dogs. *Journal of Heredity*, 69:362–365, 1978.

[23] J. M. Burger, M. Kolss, J. Pont, and T. J. Kawecki. Learning ability and longevity: a symmetrical evolutionary trade-off in *Drosophila*. *Evolution*, 62(6):1294–304, 2008.

[24] James G Burns and Frederic Mery. Transgenerational memory effect of ageing in *Drosophila*. *J Evol Biol*, 23(4):678–86, Apr 2010.

[25] W. E. Castle. Inbreeding, cross-breeding and sterility in *Drosophila*. *Science*, 23(578):153, 1906.

[26] S. B. Chandra, J. S. Hosler, and B. H. Smith. Heritable variation for latent inhibition and its correlation with reversal learning in honeybees (*Apis mellifera*). *J Comp Psychol*, 114(1):86–97, 2000.

[27] B. Charlesworth and D. Charlesworth. The genetic basis of inbreeding depression. *Genet Res*, 74(3):329–40, 1999.

[28] D Charlesworth and B Charlesworth. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.*, 18:237–268, 1987.

[29] D. Charlsworth and B. Charlsworth. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.*, 18:237–268, 1987.

[30] C C Cockerham and B S Weir. Quadratic analyses of reciprocal crosses. *Biometrics*, 33(1):187–203, Mar 1977.

[31] P. Crnokrak and S. C. Barrett. Perspective: purging the genetic load: a review of the experimental evidence. *Evolution*, 56(12):2347–58, 2002.

[32] P. Crnokrak and D. A. Roff. Inbreeding depression in the wild. *Heredity*, 83 (Pt 3):260–70, 1999.

[33] J Crow and M Kimura. *An Introduction to Population Genetics Theory*. Harper and Row: New York, 1970.

[34] J. F. Crow. *Basic concepts in population, quantitative, and evolutionary genetics*. 1986.

[35] J. P. Cunningham and S. A. West. How host plant variability influences the advantages to learning: a theoretical model for oviposition behaviour in *Lepidoptera*. *J Theor Biol*, 251(3):404–10, 2008.

[36] Etienne Danchin, Simon Blanchet, Frédérick Mery, and Richard H Wagner. Do invertebrates have culture? *Commun Integr Biol*, 3(4):303–5, Jul 2010.

[37] C R Darwin. *Letter no. 607, from Charles Darwin to the Gardener's CHronicle, published 21st August 1841. In The Correspondance of Charles Darwin, Vol2*. Cambridge University Press 1986, 1841.

[38] C. B. Davenport. Degeneration, albinism and inbreeding. *Science*, 28(718):454–455, 1908.

[39] J. R. David and M. F. Clavel. Intéraction entre le génotype et le milieu d'élevage, conséquences sur les caractéristiques de la drosophile. *Bull. Biol. Fr. Belg.*, 99:369–378, 1965.

[40] J. R. David, P. Gibert, H. Legout, G. Petavy, P. Capy, and B. Moreteau. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity*, 94(1):3–12, 2005.

[41] Ronald L Davis. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. *Annu Rev Neurosci*, 28:275–302, 2005.

[42] Thomas Degen, Christine Dillmann, Frédéric Marion-Poll, and Ted C J Turlings. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol*, 135(4):1928–38, Aug 2004.

[43] H. W. Deng, Y. X. Fu, and M. Lynch. Inferring the major genomic mode of dominance and overdominance. *Genetica*, 102-103(1-6):559–67, 1998.

[44] M A DeRose and D A Roff. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution*, 53:1288–1292, 1999.

[45] D. A. Dewsbury, J. M. Oglesby, S. L. Shea, and J. L. Connor. Inbreeding and copulatory behavior in house mice: a further consideration. *Behav Genet*, 9(3):151–63, 1979.

[46] T. Dobzhansky and N. P. Spassky. Genetic drift and natural selection in experimental populations of *Drosophila pseudoobscura*. *Proc Natl Acad Sci U S A*, 48:148–56, 1962.

[47] Y. Dudai, Y. N. Jan, D. Byers, W. G. Quinn, and S. Benzer. dunce, a mutant of *Drosophila* deficient in learning. *Proc Natl Acad Sci U S A*, 73(5):1684–8, 1976.

[48] M. R. Dudash and D. E. Carr. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature*, 393:682–684, 1998.

[49] R. Dukas. *Cognitive Ecology*. The university of CHicago Press, 1998.

[50] R. Dukas. Costs of memory: ideas and predictions. *J Theor Biol*, 197(1):41–50, 1999.

[51] R. Dukas. Evolutionary biology of animal cognition. *Annu. Rev. Evol. Ecol. Sys.*, 35:347–374, 2004.

[52] R. Dukas. Evolutionary biology of insect learning. *Annu Rev Entomol*, 53:145–60, 2008.

[53] R Dukas. *Learning: mechanisms, ecology and evolution. In: Cognitive Ecology II.* Chicago: University of Chicago Press, 2009.

[54] R. Dukas and E. A. Bernays. Learning improves growth rate in grasshoppers. *Proc Natl Acad Sci U S A*, 97(6):2637–40, 2000.

[55] A. S. Dunlap and D. W. Stephens. Components of change in the evolution of learning and unlearned preference. *Proc Biol Sci*, 276(1670):3201–8, 2009.

[56] E M East. Inbreeding in corn. *Reports of the Connecticut Agricultural Experiments Station for 1907*, pages 419–428, 1908.

[57] A. C. Edwards, J. F. Ayroles, E. A. Stone, M. A. Carbone, R. F. Lyman, and T. F. Mackay. A transcriptional network associated with natural variation in *Drosophila* aggressive behavior. *Genome Biol*, 10(7):R76, 2009.

[58] Alexis C Edwards and Trudy F C Mackay. Quantitative trait loci for aggressive behavior in *Drosophila melanogaster*. *Genetics*, 182(3):889–97, Jul 2009.

[59] A Eklund. The effects of inbreeding on aggression in wild male house mice (*Mus domesticus*). *Behaviour*, 133:883–901, 1996.

[60] D S Falconer and T Mackay. *Introduction to Quantitative Genetics, Ed. 4.* Longman Scientific and Technical, Harlow, Essex, 1996.

[61] R. Feil, F. Hofmann, and T. Kleppisch. Function of cgmp-dependent protein kinases in the nervous system. *Rev Neurosci*, 16(1):23–41, 2005.

[62] M D Fellowes, A R Kraaijeveld, and H C Godfray. Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc Biol Sci*, 265(1405):1553–8, Aug 1998.

[63] R A Fisher. The correlation between relatives on the supposition of mendelian inheritance. *Transactions of the Royal Society of Edinburgh*, 52:399–433, 1918.

[64] Jonathan Flint and Trudy F C Mackay. Genetic architecture of quantitative traits in mice, flies, and humans. *Genome Res*, 19(5):723–33, May 2009.

[65] J L Fuller and W R Thompson. *Foundatinos of Behavios Genetics*. St Louis, MO: Mosby.

[66] J A Gallardo and R Neira. Environmental dependence of inbreeding depression in cultured coho salmon (*Oncorhynchus kisutch*): aggressiveness, dominance and intraspecific competition. *Heredity*, 95(6):449–56, Dec 2005.

[67] J H Gillespie and M Turelli. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics*, 121(1):129–38, Jan 1989.

[68] S. Glemin. How are deleterious mutations purged? drift versus nonrandom mating. *Evolution*, 57(12):2678–87, 2003.

[69] B Griffing. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, 9:463–493, 1956.

[70] J B S Haldane and S D Jayakar. Polymorphism due to selection of varying direction. *Journal of Genetics*, 58:237–242, 1963.

[71] S. T. Harbison, M. A. Carbone, J. F. Ayroles, E. A. Stone, R. F. Lyman, and T. F. Mackay. Co-regulated transcriptional networks contribute to natural genetic variation in *Drosophila* sleep. *Nat Genet*, 41(3):371–5, 2009.

[72] Susan T Harbison, Trudy F C Mackay, and Robert R H Anholt. Understanding the neurogenetics of sleep: progress from *Drosophila*. *Trends Genet*, 25(6):262–9, Jun 2009.

[73] K. T. Harker and I. Q. Whishaw. Place and matching-to-place spatial learning affected by rat inbreeding (dark-agouti, fischer 344) and albinism (wistar, sprague-dawley) but not domestication (wild rat vs. long-evans, fischer-norway). *Behav Brain Res*, 134(1-2):467–77, 2002.

[74] P. W. Hedrick. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity*, 73 (Pt 4):363–72, 1994.

[75] PW Hedrick. Antagonistic pleiotropy and genetic polymorphism: a perspective. *Heredity*, 82:126–133, 1999.

[76] J. K. Hewitt, D. W. Fulker, and C. A. Hewitt. Genetic architecture of olfactory discriminative avoidance conditioning in *Drosophila melanogaster*. *J Comp Psychol*, 97(1):52–8, 1983.

[77] A A Hoffmann and P A Parsons. The analysis of quantitative variation in natural populations with isofemale strains. *Génét Sél Évol*, 20:87–98, 1988.

[78] F. Hofmann, R. Feil, T. Kleppisch, and J. Schlossmann. Function of cgmp-dependent protein kinases as revealed by gene deletion. *Physiol Rev*, 86(1):1–23, 2006.

[79] D. Houle. Comparing evolvability and variability of quantitative traits. *Genetics*, 130(1):195–204, 1992.

[80] K. A. Hughes. The inbreeding decline and average dominance of genes affecting male life-history characters in *Drosophila melanogaster*. *Genet Res*, 65(1):41–52, 1995.

[81] Jacob Höglund, Stuart B Piertney, Rauno V Alatalo, Johan Lindell, Arne Lundberg, and Pekka T Rintamäki. Inbreeding depression and male fitness in black grouse. *Proc Biol Sci*, 269(1492):711–5, Apr 2002.

[82] M. Imhof, B. Harr, G. Brem, and C. Schlotterer. Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. *Mol Ecol*, 7(7):915–7, 1998.

[83] G. Isabel, A. Pascual, and T. Preat. Exclusive consolidated memory phases in *Drosophila*. *Science*, 304(5673):1024–7, 2004.

[84] T. D. Johnston. Selective costs and benefits in the evolution of learning. *Advances in the Study of Behavior*, 12:65–106, 1982.

[85] Katherine W Jordan, Mary Anna Carbone, Akihiko Yamamoto, Theodore J Morgan, and Trudy F C Mackay. Quantitative genomics of locomotor behavior in *Drosophila melanogaster*. *Genome Biol*, 8(8):R172, 2007.

[86] M. Joron and P. M. Brakefield. Captivity masks inbreeding effects on male mating success in butterflies. *Nature*, 424(6945):191–4, 2003.

[87] E R Kandel, T Abrams, L Bernier, T J Carew, R D Hawkins, and J H Schwartz. Classical conditioning and sensitization share aspects of the same molecular cascade in *Aplysia*. *Cold Spring Harb Symp Quant Biol*, 48 Pt 2:821–30, 1983.

[88] D Karan, J-P Morin, Gravot E, B Moreteau, and J R David. Body size reaction norms in *Drosophila melanogaster*: temporal stability and genetic architecture in a natural population. *Genetics selection evolution*, 31:491–508, 1999.

[89] K. R. Kaun, M. Chakaborty-Chatterjee, and M. B. Sokolowski. Natural variation in plasticity of glucose homeostasis and food intake. *J Exp Biol*, 211(Pt 19):3160–6, 2008.

[90] K. R. Kaun, T. Hendel, B. Gerber, and M. B. Sokolowski. Natural variation in drosophila larval reward learning and memory due to a cgmp-dependent protein kinase. *Learn Mem*, 14(5):342–9, 2007.

[91] T J Kawecki. Evolutionary ecology of learning: insights from fruit flies. *Population Ecology*, 52:15–25, 2010.

[92] T. J. Kawecki and F. Mery. Genetically idiosyncratic responses of *Drosophila melanogaster* populations to selection for improved learning ability. *J Evol Biol*, 19(4):1265–74, 2006.

[93] L. F. Keller and D. M. Waller. Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, 17(5):230–241, 2002.

[94] E Kisdi and S A H GERITZ. Adaptive dynamics in allele space: Evolution of genetic polymorphism by small mutations in a heterogeneous environment. *Evolution*, 53:993–1008, 1999.

[95] T. Kleppisch and R. Feil. cgmp signalling in the mammalian brain: role in synaptic plasticity and behaviour. *Handb Exp Pharmacol*, (191):549–79, 2009.

[96] M. Kolss and T. J. Kawecki. Reduced learning ability as a consequence of evolutionary adaptation to nutritional stress in *Drosophila melanogaster*. *Ecological Entomology*, 33(5):583–588, 2008.

[97] M. Kolss, A. R. Kraaijeveld, F. Mery, and T. J. Kawecki. No trade-off between learning ability and parasitoid resistance in *Drosophila melanogaster*. *J Evol Biol*, 19(4):1359–63, 2006.

[98] R. C. Lacy and J. D. Ballou. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution*, 52(3):900–909, 1998.

[99] R Lande. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics*, 94(1):203–15, Jan 1980.

[100] R Lande and D. W. Schemske. The evolution of self-fertilization and inbreeding depression in plants. i. genetic models. *Evolution*, 39(1):24–40, 1985.

[101] B. D. Latter, J. C. Mulley, D. Reid, and L. Pascoe. Reduced genetic load revealed by slow inbreeding in *Drosophila melanogaster*. *Genetics*, 139(1):287–97, 1995.

[102] BDH. Latter and J. A. Sved. A reevaluation of data from competitive tests shows high levels of heterosis in *Drosophila melanogaster*. *Genetics*, 137:509–511, 1994.

[103] S B Laughlin. Energy as a constraint on the coding and processing of sensory information. *Curr Opin Neurobiol*, 11(4):475–80, Aug 2001.

[104] H Levene. Genetic equilibrium when more than one ecological niche is available. *The American Naturalist*, 87:331–333, 1953.

[105] W J Lewis and J H Tumlinson. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature*, 331:257–259, 1988.

[106] M S Livingstone, P P Sziber, and W G Quinn. Loss of calcium/calmodulin responsiveness in adenylate cyclase of rutabaga, a *Drosophila* learning mutant. *Cell*, 37(1):205–15, May 1984.

[107] K. L. Lofdahl, M. Holliday, and J. Hirsch. Selection for conditionability in *Drosophila melanogaster*. *J Comp Psychol*, 106(2):172–83, 1992.

[108] M. Lynch, J. Conery, and P. W. Hedrick. Mutation accumulation and the extinction of small populations. *Am. Nat.*, 146:489–518, 1995.

[109] M. Lynch and B. Wash. *Genetics and Ananlysis of Quantitative Traits*. Sinauer Associates, 1998.

[110] E. J. Lyons, A. J. Frodsham, L. Zhang, A. V. Hill, and W. Amos. Consanguinity and susceptibility to infectious diseases in humans. *Biol Lett*, 2009.

[111] T F Mackay. The genetic architecture of quantitative traits. *Annu Rev Genet*, 35:303–39, 2001.

[112] T. F. Mackay, E. A. Stone, and J. F. Ayroles. The genetics of quantitative traits: challenges and prospects. *Nat Rev Genet*, 10(8):565–77, 2009.

[113] T. F. C. Mackay. A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*. *Genet. Res.*, 47:59–70, 1985.

[114] Trudy F C Mackay. Mutations and quantitative genetic variation: lessons from *Drosophila*. *Philos Trans R Soc Lond B Biol Sci*, 365(1544):1229–39, Apr 2010.

[115] Eamonn B Mallon, Axel Brockmann, and Paul Schmid-Hempel. Immune response inhibits associative learning in insects. *Proc Biol Sci*, 270(1532):2471–3, Dec 2003.

[116] S. W. Margulis. Relationships among parental inbreeding, parental behaviour and offspring viability in oldfield mice. *Anim Behav*, 55(2):427–38, 1998.

[117] S. W. Margulis and J. Altmann. Behavioural risk factors in the reproduction of inbred and outbred oldfield mice. *Anim Behav*, 54(2):397–408, 1997.

[118] M. Mariette, J.L. Kelley, R. Brooks, and J.P. Evans. The effects of inbreeding on male courtship ehaviour and coloration in guppies. *Ethology*, 112(8):807–814, 2006.

[119] E Mayr. Behavior programs and evolutionary strategies. *American scientist*, 62:650–659, 1974.

[120] T R McGuire and J Hirsh. Behavior genetic analysis of *Formia regina*: conditioning, reliable individual differences, and selection. *Proc. Natl. Acad. Sci. USA.*, 74:5193–5197, 1977.

[121] C. McNeely and M. C. Singer. Contrasting the roles of learning in butterflies foraging for nectar and oviposition sites. *Animal Behaviour*, 61(4):847–852, 2000.

[122] S Meagher, D J Penn, and W K Potts. Male-male competition magnifies inbreeding depression in wild house mice. *Proc Natl Acad Sci U S A*, 97(7):3324–9, Mar 2000.

[123] Lisa M Meffert, Sara K Hicks, and Jennifer L Regan. Nonadditive genetic effects in animal behavior. *Am Nat*, 160 Suppl 6:S198–213, Dec 2002.

[124] L.M. Meffert and E.H. Bryant. Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. *Evolution*, 45(2):293–306, 1991.

[125] R Menzel, J Erber, and T Masuhr. *Learning and memory in the honeybee. In L. Barton-Browne (ed): Experimental analysis of insect behaviour*. Berlin: Springer, 1974.

[126] F. Mery, A. T. Belay, A. K. So, M. B. Sokolowski, and T. J. Kawecki. Natural polymorphism affecting learning and memory in *Drosophila*. *Proc Natl Acad Sci U S A*, 104(32):13051–5, 2007.

[127] F Mery and J Burns. Behavioural plasticity: an interaction between evolution and experience. *Evolutionary Ecology*, 24:571–583, 2010.

[128] F. Mery and T. J. Kawecki. Experimental evolution of learning ability in fruit flies. *Proc Natl Acad Sci U S A*, 99(22):14274–9, 2002.

[129] F. Mery and T. J. Kawecki. A fitness cost of learning ability in *Drosophila melanogaster*. *Proc Biol Sci*, 270(1532):2465–9, 2003.

[130] F. Mery and T. J. Kawecki. A cost of long-term memory in *Drosophila*. *Science*, 308(5725):1148, 2005.

[131] F Mery, J Pont, T Preat, and T J Kawecki. Experimental evolution of olfactory memory in *Drosophila melanogaster*. *Physiol Biochem Zool*, 80(4):399–405, 2007.

[132] F. Mery, S. A. Varela, E. Danchin, S. Blanchet, D. Parejo, I. Coolen, and R. H. Wagner. Public versus personal information for mate copying in an invertebrate. *Curr Biol*, 19(9):730–4, 2009.

[133] T Michalczyk, A L Millard, O Y Martin, A J Lumley, C Emerson, T Chapman, and M J G Gage. Inbreeding promotes female promiscuity. *Science*, 333(6050):1739–42, Sep 2011.

[134] R. Milkmann and R. R. Zeitler. Concurrent multiple paternity in natural and laboratory populations of *Drosophila melanogaster*. *Genetics*, 78(4):1191–3, 1974.

[135] P. S. Miller, J. Glasner, and P. W. Hedrick. Inbreeding depression and male-mating behavior in *Drosophila melanogaster*. *Genetica*, 88(1):29–36, 1993.

[136] J Moore and A Rauf. Are dispersal and inbreeding avoidance related? *Animal Behaviour*, 32:94–112, 1984.

[137] J. V. Neel, W. J. Schull, M. Yamamoto, S. Uchida, T. Yanase, and N. Fujiki. The effects of parental consanguinity and inbreeding in hirado, japan. ii. physical development, tapping rate, blood pressure, intelligence quotient, and school performance. *Am J Hum Genet*, 22(3):263–86, 1970.

[138] V Nepoux, C R Haag, and T J Kawecki. Effects of inbreeding on aversive learning in *Drosophila*. *J Evol Biol*, 23(11):2333–45, Nov 2010.

[139] E. Nevo, E. Rashkovetsky, T. Pavlicek, and A. Korol. A complex adaptive syndrome in drosophila caused by microclimatic contrasts. *Heredity*, 80 (Pt 1):9–16, 1998.

[140] S J O'Brien and R J MacIntyre. An analysis of gene-enzyme variability in natural populations of *Drosophila melanogaster* and *D. simulans*. *The American Naturalist*, 103:97–113, 1969.

[141] L. Ollivier. *Eléments de génétique quantitative*. INRA, 2002.

[142] K. A. Osborne, A. Robichon, E. Burgess, S. Butland, R. A. Shaw, A. Coulthard, H. S. Pereira, R. J. Greenspan, and M. B. Sokolowski. Natural behavior polymorphism due to a cgmp-dependent protein kinase of *Drosophila*. *Science*, 277(5327):834–6, 1997.

[143] D R Papaj and R J Prokopy. Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, 34:315–350, 1989.

[144] D R Papaj and L E M Vet. Odor learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *Journal of Chemical Ecology*, 16:3137–3150, 1990.

[145] C. Parmesan, M. C. Singer, and I. Harris. Absence of adaptive learning from the oviposition foraging behaviour of a checkerspot butterfly. *Animal Behaviour*, 50(1):161–175, 1995.

[146] I P Pavlov. *Conditioned reflexes.* 1927.

[147] Y. Peng, W. Xi, W. Zhang, K. Zhang, and A. Guo. Experience improves feature extraction in *Drosophila*. *J Neurosci*, 27(19):5139–45, 2007.

[148] H. S. Pereira and M. B. Sokolowski. Mutations in the larval foraging gene affect adult locomotory behavior after feeding in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*, 90(11):5044–6, 1993.

[149] T Prout. Some relationships between density-dependent selection and density-dependent population growth. *Evol Biol*, 13, 1-68.

[150] A. Pusey and M. Wolf. Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, 11(5):201–206, 1996.

[151] R Pérez-Maluf, L Kaiser, E Wajnberg, Y Carton, and M H Pham-Delègue. Genetic variability of conditioned probing responses to a fruit odor in *Leptopilina boulardi* (hymenoptera: Eucoilidae), a *Drosophila* parasitoid. *Behav Genet*, 28(1):67–73, Jan 1998.

[152] W. G. Quinn and Y. Dudai. Memory phases in *Drosophila*. *Nature*, 262(5569):576–7, 1976.

[153] W. G. Quinn, W. A. Harris, and S. Benzer. Conditioned behavior in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*, 71(3):708–12, 1974.

[154] W Reichardt and H Wenking. Optical detection and fixation of objects by fixed flying flies. *Naturwissenschaften*, 56(8):424–5, Aug 1969.

[155] J. Reid, P. Arcese, A. E. Cassidy, A. Marr, J. M. Smith, and L. Keller. Hamilton and zuk meet heterozygosity? song repertoire size indicates inbreeding and immunity in song sparrows (*Melospiza melodia*). *Proc Biol Sci*, 272(1562):481–7, 2005.

[156] M. Reif, K. E. Linsenmair, and M. Heisenberg. Evolutionary significance of courtship conditioning in *Drosophila melanogaster*. *Animal Behaviour*, 63(1):143–155, 2002.

[157] R A Rescorla. Behavioral studies of pavlovian conditioning. *Annu Rev Neurosci*, 11:329–52, 1988.

[158] Carolyn E Riddell and Eamonn B Mallon. Insect psychoneuroimmunology: immune response reduces learning in protein starved bumblebees (*Bombus terrestris*). *Brain Behav Immun*, 20(2):135–8, Mar 2006.

[159] A. Robertson. The effect of inbreeding on the variation due to recessive genes. *Genetics*, 37(2):189–207, 1952.

[160] A Robertson. Selection in animals: Synthesis. *Cold Spring Harbor Symp. Quant. Biol.*, 20:225–229, 1955.

[161] D A Roff and D J Fairbairn. The evolution of trade-offs: where are we? *J Evol Biol*, 20(2):433–47, Mar 2007.

[162] Stephanie M Rollmann, Ping Wang, Priya Date, Steven A West, Trudy F C Mackay, and Robert R H Anholt. Odorant receptor polymorphisms and natural variation in olfactory behavior in *Drosophila melanogaster*. *Genetics*, 186(2):687–97, Oct 2010.

[163] L Ronnegard, X Shen, and M Alam. hglm: A package for fitting hierarchical generalized linear models. *The R Journal*, 2010.

[164] M R Rose. Antagonistic pleiotropy, dominance, and genetic variation. *Heredity*, 48:63–78, 1982.

[165] M R Rose and B Charlesworth. Genetics of life history in *Drosophila melanogaster*. i. sib analysis of adult females. *Genetics*, 97(1):173–86, Jan 1981.

[166] I Rudan, D Rudan, H Campbell, Z Biloglav, R Urek, M Padovan, L Sibbett, B Janicijevic, N Smolej Narancic, and P Rudan. Inbreeding and learning disability in croatian island isolates. *Coll Antropol*, 26(2):421–8, Dec 2002.

[167] W Rumball, I R Franklin, R Frankham, and B L Sheldon. Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics*, 136(3):1039–49, Mar 1994.

[168] I Scharf, I Filin, and O Ovadia. A trade-off between growth and starvation endurance in a pit-building antlion. *Oecologia*, 160(3):453–60, Jun 2009.

[169] W J Schull and J V Neel. The effects of parental consanguinity and inbreeding in hirado, japan. v. summary and interpretation. *Am J Hum Genet*, 24(4):425–53, Jul 1972.

[170] P M Sharp. The effect of inbreeding on competitive male-mating ability in *Drosophila melanogaster*. *Genetics*, 106(4):601–12, Apr 1984.

[171] S A Shaver, C J Varnam, A J Hilliker, and M B Sokolowski. The foraging gene affects adult but not larval olfactory-related behavior in *Drosophila melanogaster*. *Behav Brain Res*, 95:23–9, 1998.

[172] S J Shettleworth. *Cognition, Evolution and Behaviour*. 1998.

[173] G H Shull. The composition of a field of maize. *Reports of the American Breeders Association*, 4:296–301, 1908.

[174] R W Siegel and J C Hall. Conditioned responses in courtship behavior of normal and mutant *Drosophila*. *Proc Natl Acad Sci U S A*, 76(7):3430–4, Jul 1979.

[175] L W Simmons. *Sperm Competition and Its Evolutionary Consequences in the Insects*. Princeton University Press, 2001.

[176] M J Simmons and J F Crow. Mutations affecting fitness in *Drosophil*a populations. *Annu Rev Genet*, 11:49–78, 1977.

[177] B F Skinner. *Science and human behavior.* 1953.

[178] M B Sokolowski. Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behav Genet*, 10:291–302, 1980.

[179] D M Spencer. *Yeast in natural and artificial habitats.* Springer, 1997.

[180] G F Sprague and L A Tatum. General vs. specific combining ability in single crosses of corn. *J. Am. Soc. Agron*, 34:923–32, 1942.

[181] S Stearns. The evolutionary significance of phenotypic plasticity: phenotypic sources of variation among organisms can be described by developmental switches and reaction norms. *BioScience*, 36:436–446, 1989.

[182] S C Stearns. *The evolution of life histories.* Oxford: Oxford university press, 1992.

[183] D W Stephens. Change, regularity and value in the evolution of animal learning. *International Society for Behavioral Ecology*, 2:77–89, 1991.

[184] R Development Core Team. *R: a language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria, 2004. ISBN 3-900051-00-3.

[185] B L Tempel, N Bonini, D R Dawson, and W G Quinn. Reward learning in normal and mutant *Drosophila*. *Proc Natl Acad Sci U S A*, 80:1482–6, 1983.

[186] T Tully, T Preat, S C Boynton, and M Del Vecchio. Genetic dissection of consolidated memory in *Drosophila*. *Cell*, 79(1):35–47, Oct 1994.

[187] C van Oosterhout, R E Trigg, G R Carvalho, A E Magurran, L Hauser, and P W Shaw. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. *J Evol Biol*, 16(2):273–81, Mar 2003.

[188] Nicolas Vodovar, Marisa Vinals, Peter Liehl, Alan Basset, Jeril Degrouard, Paul Spellman, Frédéric Boccard, and Bruno Lemaitre. Drosophila host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proc Natl Acad Sci U S A*, 102(32):11414–9, Aug 2005.

[189] S Waddell and W G Quinn. Flies, genes, and learning. *Annu Rev NeuroscI*, 24:1283–309, 2001.

[190] S Waddell and W G Quinn. Neurobiology. learning how a fruit fly forgets. *Science*, 293(5533):1271–2, Aug 2001.

[191] J Wang, W G Hill, D Charlesworth, and B Charlesworth. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet Res*, 74(2):165–78, Oct 1999.

[192] Ping Wang, Richard F Lyman, Trudy F C Mackay, and Robert R H Anholt. Natural variation in odorant recognition among odorant-binding proteins in *Drosophila melanogaster*. *Genetics*, 184(3):759–67, Mar 2010.

[193] K Mathias Wegner, Martin Kalbe, Helmut Schaschl, and Thorsten B H Reusch. Parasites and individual major histocompatibility complex diversity–an optimal choice? *Microbes Infect*, 6(12):1110–6, Oct 2004.

[194] Glenn E Weisfeld, Tiffany Czilli, Krista A Phillips, James A Gall, and Cary M Lichtman. Possible olfaction-based mechanisms in human kin recognition and inbreeding avoidance. *J Exp Child Psychol*, 85(3):279–95, Jul 2003.

[195] R Wolf and M Heisenberg. On the fine structure of yaw torque in visual flight orientation of *Drosophila melanogaster*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 140:69–80, 1980.

[196] R Wolf and M Heisenberg. Basic organization of operant behavior as revealed in *Drosophila* flight orientation. *J Comp Physiol A*, 169(6):699–705, Dec 1991.

[197] D C Wood. Habituation in *Stentor*: a response-dependent process. *J Neurosci*, 8(7):2248–53, Jul 1988.

[198] G Wricke and W E Weber. *Quantitative genetics and selection in plant breeding*. de Gruyter, Berlin, 1986.

[199] A J Wright. Diallel designs, analyses, and reference populations. *Heredity*, 54 (Pt 3):307–11, Jun 1985.

[200] S Wright. Systems of mating. i. the biometric relations between parent and offspring. *Genetics*, 6(2):111–23, Mar 1921.

[201] S Wright. Physiological and evolutionary theories of dominance. *Am. Nat.*, 67:24–53, 1934.

[202] S Wright. *Evolution and the Genetics of Populations, Vol 3, Experimental Results, and Evolutionary Deductions*. University of Chicago Press, 1977.

[203] Alexandra Yurkovic, Oulu Wang, Alo C Basu, and Edward A Kravitz. Learning and memory associated with aggression in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*, 103(46):17519–24, Nov 2006.

Acknowledgements

I would like to thank all the people who encouraged and helped me during my PhD work. First of all, special thanks to Tad Kawecki, for accepting me in his group and supporting me all these years. I also would like to acknowledge Frederic Mery and Mike Ritchie who accepted to be my examiners, and Nicolas Mermod who accepted to be the president of the thesis committee.

I would also specially thank Aurélie Babin, collaborating with you was great, I will even keep a good memory of full nights working in the lab! Many thanks to Arnaud Le Rouzic, for his patience explaining statistics, and his kindness answering email questions even during weekend nights, and to Christoph Haag for his kind help and support.

Grateful thanks to Severine Büchel, Geraldine Mudavadi, Vassilissa Dolivo-Beuret, Sophie Cotting, for their help in the lab, the endless days counting eggs and testing flies in the dark room. Many thanks also to Marco Zini, Frida Rosengren, Ute Friedrich, Cindy Burger, Lusia Sygnarski and all the students helpers of the kitchen for their great technical help.

Very warm thanks to all the members of the lab, for the discussions, great working environement and the friendship. My office mates I did not mention yet, Vukasin Zrelec (when are we going in Croatia again?), Yvan Mateus, Elise Dirand, Ludivine Strambini and all the other members of the lab, Brian Hollis, Roshan Vijendravarma, Sunitha Narasimha, Pierre Millon, Joep Burger, Juliette Pont, Munjong Kolss, Stephanie Rion, Evelin Hürlimann, Ana Rita Gonçalves and all other I may have forgotten...

Thanks to the members of Ecology and Evolution departments of Fribourg and Lausanne who shared with me time and discussion, I enjoyed this time a lot.

I also would like to very warmly thank my parents, my sister, and my friends and family for being there and supporting me. And I would like to express a very special thank to Luc, for being here with me, for his love, for everything.

And finally, I would like to thank and present my apologies to all the flies which contributed to this work!

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Year : 2011

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Originally published at : Thesis, University of Lausanne

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