Male cognitive performance declines in the absence of sexual selection

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Abstract
Sexual selection is responsible for the evolution of male ornaments and armaments, but its role in the evolution of cognition—the ability to process, retain, and use information—is largely unexplored. Because successful courtship is likely to involve processing information in complex, competitive sexual environments, we hypothesized that sexual selection contributes to the evolution and maintenance of cognitive abilities in males. To test this, we removed mate choice and mate competition from experimental populations of *Drosophila melanogaster* by enforcing monogamy for over 100 generations. Males evolved under monogamy became less proficient than polygamous control males at relatively complex cognitive tasks. When faced with one receptive and several unreceptive females, polygamous males quickly focused on receptive females, whereas monogamous males continued to direct substantial courtship effort towards unreceptive females. As a result, monogamous males were less successful in this complex setting, despite being as quick to mate as their polygamous counterparts with only one receptive female. This diminished ability to use past information was not limited to the courtship context: monogamous males (but not females) also showed reduced aversive olfactory learning ability. Our results provide direct experimental evidence that the intensity of sexual selection is an important factor in the evolution of male cognitive ability.

Keywords: sexual selection, cognition, learning, experimental evolution, courtship, Drosophila

Introduction

Sexual selection is usually not considered a major force driving the evolution of cognition, in particular in animals with stereotyped, genetically-determined courtship [1]. Yet, males of many species are faced with a complex and competitive sexual environment containing both male competitors and females of varying quality and receptivity. For example, in *Drosophila* mating takes place in aggregations on food sources, where flies also feed and lay eggs. Females of several species are often present, only a fraction of conspecific females are receptive at any time, and these receptive females are often greatly outnumbered by males searching for mating opportunities. The ability to locate and focus courtship efforts on receptive and fertile conspecific females is thus a crucial determinant of male reproductive success. These abilities involve processing complex sensory information and are known to rely in part on learning [2-6]. We therefore hypothesized that sexual selection contributes to the maintenance of such cognitive abilities in males.

To test this hypothesis we imposed strict monogamy on three replicate populations of the fruit fly *Drosophila melanogaster* for over 100 generations by randomly pairing males and females, thus eliminating all modalities of sexual selection, including competition for mates, mate choice and sexual conflict. Three polygamous control populations which experienced sexual selection were maintained in parallel. Any adaptations in males that aid competitive mating success, including the ability to differentiate between receptive and unreceptive females or persuade females to mate, should be advantageous under polygamy but virtually irrelevant under this monogamy regime. Therefore, if cognitive abilities that contribute to sexual success carry any cost they should decline under monogamy owing to the action of natural selection. Even without costs, a decline is expected due to mutation accumulation and genetic drift. Consistent with these predictions, we show that cognitive performance of males from monogamous populations is reduced relative to polygamous control males, both in sexual and non-sexual contexts. This rapid evolutionary decay points to a fundamental role of sexual selection in the maintenance of cognitive performance.

Results

We first determined whether males from monogamous populations have reduced competitive reproductive success relative to polygamous males. Groups of five sexually naive males from either a monogamous or a polygamous population were allowed to compete with five *ebony* males for mating opportunities with five *ebony* females. The *ebony* flies used in our experiment come from an independent population with an uncontrolled polygamous mating system. Because these flies have dark coloration caused by a recessive allele, any wild type progeny produced by *ebony* females in this assay must be sired by males from the focal experimental populations. In this competitive setting, males from monogamous populations had greatly reduced reproductive success relative to polygamous males (figure 1, $F_{1,4} = 25.10$, $p < .01$).

This difference in male sexual success might be due to females being more reluctant to mate with monogamous males (e.g. because the males are less attractive or court less vigorously). However, when individual males were allowed to court and mate

with a single receptive female, there was no difference between selection regimes in the time to copulation (figure 2, $t_4 = 0.06$, $p = .95$). While this does not necessarily mean that females would exhibit no preference in a choice situation [7], it does indicate no gross difference in male attractiveness to females. Further, males from the two selection regimes did not differ in their locomotor activity in two assays (climbing response to shock: figure S1a, $F_{1,4} = 0.44$, $p = .54$, overall locomotion: figure S1b, $F_{1,4} = 0.95$, $p = .38$), indicating that monogamous males are not less active or mobile than polygamous males.

We thus hypothesized that the lower competitive reproductive success of monogamous males is due in part to the challenge presented by the presence of multiple females of varying levels of receptivity. To test this, we quantified time to copulation of single males faced with one receptive (virgin) female accompanied by either one or five unreceptive, previously mated females. Males from both selection regimes took longer to achieve mating when five rather than one unreceptive females were present (figure 3a,b, $t_4 = 4.67$, $p < .01$). This indicated that the presence of multiple unreceptive females interfered with male success with a receptive female. Furthermore, this interference had a greater effect on males from monogamous populations than it did on males from polygamous populations. Whereas both types of males achieved copulation equally rapidly when only one unreceptive female was present (figure 3a, selection regime effect: $t_4 = 0.68$, $p = .53$), monogamous males were slower than polygamous males when five unreceptive females were present (figure 3b, selection regime effect: $t_4 = -4.02$, $p = .02$). This effect was large—the median monogamous male took 19 minutes (75%) longer to achieve copulation than the median polygamous male.

In order to shed light on the mechanism behind this difference, we again confronted single males with one receptive female and five unreceptive females and observed their behavior during the first 20 minutes of interaction. Every minute we recorded whether or not the male was courting and, if so, whether the courtship was directed at the receptive female. Males from both selection regimes courted more as time passed (figure 4a, time effect: $F_{1,181} = 57{,}75$, $p < .0001$), but selection regime did not affect overall courtship intensity (selection regime effect: $F_{1,4} = 0.35$, $p = .59$, selection regime x time interaction: $F_{1,181} = 0.20$, $p = .66$). However, even though over time males from both selection regimes increasingly focused their courtship effort on the receptive female, (figure 4b; monogamous: $F_{1,91} = 12.06$, $p < .001$, polygamous: $F_{1,90} = 55.95$, $p < .0001$), this improvement in focus was more pronounced in polygamous males than monogamous males (selection regime x time interaction, $F_{1,181} = 8.30$, $p < .01$). By the end of the 20 minute observational period, 88% of polygamous males were courting the receptive female, versus only 62% of monogamous males.

The increasing focus of courtship activity on the receptive female indicates that the ability to discriminate between receptive and unreceptive females improves with experience. This is consistent with previous research which has shown that male ability to discriminate against unreceptive females relies in part on associative learning, whereby olfactory cues emitted by unreceptive females are associated with failed courtship [3, 8-10]. Does the poorer focus of courtship on the receptive female shown by the monogamous males reflect their poorer olfactory learning, and if so, does the

difference extend to non-sexual contexts? We addressed this question with a Pavlovian conditioning assay [11] that challenged groups of flies to form an association between an odor and aversive mechanical shock. Because this assay could be applied to flies of either sex, it also allowed us to test if differences in learning ability between the monogamous and polygamous populations are specific to males or extend to females. Same-sex groups of flies were exposed to cycles of one odor presented with shock and a second odor without shock. One hour later, the flies were placed in an elevator maze and allowed to choose between the two odors for 60 seconds. We found that males from the monogamous regime indeed showed reduced learning performance in this assay relative to males from the polygamous regime (average learning scores of .17 versus .42, respectively, figure 5a, $F_{1,4} = 26.60$, $p < .01$). Importantly, no such difference was observed for females; if anything, monogamous females tended to learn slightly (but not significantly) better than polygamous females (figure 5b, selection regime effect for female data: $F_{1,4} = 3.40$, $p = .14$; sex × regime interaction for male and female data combined: $F_{1,133} = 37.12$, $p < .0001$). Neither sex differed between the selection regimes in innate responses to the odorants used in the assays (figure S2a-d; males: $F_{1,4} = 0.03$, $p = .86$, females: $F_{1,4} = 0.07$, $p = .81$), indicating that the difference in male learning performance is not due to a difference in odor perception.

Discussion

Evolution in the absence of sexual selection led to reduction in the performance of males in two relatively complex cognitive tasks: the ability to focus courtship efforts on a receptive female mixed with several unreceptive females and the ability to avoid an odor previously paired with aversive shock. While the latter task obviously relies on associative learning, the difference in the ability to focus on receptive females is also likely to reflect reduced ability of monogamous males to profit from experience. This is indicated by the faster improvement of courtship focus in polygamous than monogamous males over time and is consistent with the known role of learning in discrimination between receptive and unreceptive females [3, 8-10, 12] and between females and immature males [13]. Performance in simpler behavioral tasks—mating with a single receptive female, locomotion, and climbing response to shock—was not affected. It is possible that other aspects of cognition, for example the ability for males to discriminate between receptive and unreceptive females based on olfactory, visual, or auditory cues, could be different between males from monogamous and polygamous selection regimes. We have no evidence for such a difference, though, as the main effect—a longer time to copulation in males from monogamous populations when housed with many unreceptive females—does not show up when males are paired with one receptive and one unreceptive female. Furthermore, naïve monogamous males respond to odors as strongly as naïve polygamous males.

These declines in complex cognitive tasks evolved independently in all three replicate populations subject to monogamy, thus excluding random genetic drift as their sole cause [14]. They are also unlikely to reflect stronger inbreeding of the monogamous populations. Under our selection regimes, monogamous populations have an equivalent or greater effective population size (because of reduced variation in male mating

success) than polygamous populations, and thus are less vulnerable to the effects of inbreeding. Further, flies from monogamous populations outperform polygamous flies on measures of net reproductive output [15], which would not be expected if they were suffering from stronger inbreeding depression. Lastly, inbreeding should affect both sexes similarly, yet females did not differ between selection regimes in learning performance.

Our monogamous selection regime minimizes conflict between the sexes over mating and female reproductive effort [16] and therefore should favor less antagonistic males which harass females less. However, we show that monogamous males court as intensely as polygamous males and are also as quick to mate when paired with individual, receptive females. Furthermore, it is not clear why reduction in male harassment would lead to a diminished ability to learn. The evolutionary decline in male performance in our monogamous fly populations is therefore unlikely to have been favored as a means of reducing sexual antagonism.

The reduced male cognitive performance we see under monogamy is instead likely to be a consequence of its diminished adaptive value in the absence of male competition and female choice. The ability to learn is a costly adaptation [17-21], expected to be maintained only if the costs are exceeded by its benefits for Darwinian fitness. If the benefits diminish due to environmental change or experimental manipulation, natural selection is expected to favor reduced investment in such costly traits. Alternatively, reduced male cognitive ability could result from antagonistic pleiotropy between the sexes [22]. If alleles reducing male cognitive performance improve some aspect of female fitness, they are expected to increase in frequency once selection on males has been relaxed by enforced monogamy. Furthermore, even without trade-offs, traits that cease to be adaptive are expected to decay due to genetic drift and mutation accumulation. One might speculate that complex cognitive traits should be more prone to such decay because they involve the interaction of many components (and thus present a larger genomic target for mutations) and are more sensitive to deviations from the optimal state of those components. Consistent with this notion, olfactory learning performance in *Drosophila* is more sensitive to inbreeding than innate responses to odors [23]. We cannot discern to what extent the reduced cognitive performance in our monogamous males is due to direct selection favoring reduced investment in cognitive traits versus decay by genetic drift or mutation accumulation after selection has been relaxed [24]. In either case, our results reveal that sexual selection is a crucial force maintaining male cognitive performance in *Drosophila*.

Although our study focuses on males, female choice also involves perception and processing of complex information and, in *Drosophila,* is known to involve learning from experience [28] as well as following choices made by other females [29]. As the opportunity for female choice is eliminated in our monogamy regime, the adaptive value of mate choice-related cognitive traits might be expected to diminish for females as well as for males. It is thus remarkable that, in contrast to males, female olfactory learning performance did not decline after 100 generations under monogamy. This not only demonstrates that the learning abilities of the two sexes can diverge, but also

suggests that learning brings mate choice-independent fitness advantages to females even under simple laboratory conditions. Possibly, learning is still important for females in the context of mating under monogamy because it allows a female to learn that no other males are around and thus accept a male perceived to be of a low quality. Alternatively, because females were pooled after mating and laid eggs under a high density of 50 females per 16 cm^2 of medium surface, it is possible that learning helps females compete for food and oviposition sites. Finally, we cannot exclude that the costs of learning are simply lower for females than males.

It has been suggested that the complexity of the social environment is a major factor in the evolution of brain size and cognition, and this 'social brain hypothesis' has received empirical support (reviewed in [25]). The role of mating systems, however, is more complicated. Work looking specifically at mating systems in non-human primates [26] and bats [27] shows higher brain investment in species with relatively less male-male competition for mating opportunities. This has been interpreted as a consequence of the cognitive demands of pair bonding, non-existent in our system, along with the resources freed by reduced investment in metabolically expensive testes. The disparity between that work and our own results, where monogamous males show reduced cognitive performance, highlights the fact that the cognitive challenges imposed by different mating systems are likely to depend on taxon-specific details and differ between the sexes. For example, in some polygynous mammal species males outperform females in spatial learning tasks while such dimorphism is smaller or absent in related monogamous species [30-32]. These differences have been attributed to differences in home range rather than directly to sexual selection—males in polygynous species typically roam over much larger areas than females, while in monogamous species the home ranges of the two sexes tend to be similar [31]. While this interpretation may be correct, our study provides direct experimental evidence that sexual selection can influence the evolution of cognition independently of differences in spatial behavior by targeting those cognitive traits that aid individuals in mate competition within complex sexual environments.

Materials and methods

(a) Experimental evolution design

The fly populations used in the experiment have been described previously [15]. Briefly, a long-term laboratory population (the IV population) that was initiated from wild *D. melanogaster* captured in 1975 was subdivided into six replicate populations in 2007. In three of these populations, the opportunity for sexual selection was minimized by enforcing monogamy. In the remaining three polygamous populations, flies experienced both female choice and male-male competition every generation. All of the populations were maintained throughout the experiment with a census size of 200 individuals.

In order to enforce monogamy, each generation virgin females were randomly paired with one virgin male each and allowed to spend two days mating in vials. In contrast, in polygamous populations groups of 5 virgin females were combined with groups of 5 virgin males in vials and also allowed to spend two days mating. After two days in these vials, males from both selection regimes were discarded and females from

each replicate population were placed into two bottles, 50 females per bottle. The mated females spent the next three days laying eggs in these bottles before also being discarded. These bottles were the source of the next generation's flies, which were passed back through the experimental treatment.

(b) General assay methods

All assays were performed between 88 and 114 generations of evolution, after allowing one generation of common garden rearing in order to control for parental effects. The flies used in these assays were 4-5 days old, with ages matched to the day within all individual assays. The assays were performed in standard culture vials, always with standard 2% yeast food (water, agar [Milian CH], brewer's yeast [Migros CH], cornmeal, sucrose, and Nipagin [Sigma-Aldrich CH] present. When assays spanned more than one day, the assays were performed in balanced blocks so that the same number of measures were taken for all populations each day.

Courtship assays all took place during the morning hours between lights on at 8AM and noon. The males used were unmated except where noted. Receptive females came from the base IV population and so are equally related to all of the evolved populations.

The IVe population, established in 1992 from a spontaneous recessive *ebony* mutant repeatedly backcrossed into the IV background [33], was used as a standardized competitor in tests of male reproductive success and also as the source for unreceptive females. These *ebony* females are easily distinguished from wild type flies by body coloration, making the assays technically manageable, but are otherwise behaviorally unimpaired. In order to generate unreceptive IVe females, groups of 15 ebony males and 5 ebony female virgins were placed in vials during the evening before each experiment for mating. Females classified as unreceptive rarely mated with males in assays performed the following morning (five times total) and the rate of occurrence did not differ between selection regimes, so when this occurred the vials were discarded and no observations were retained for analyses. Likewise, if an individual fly died or escaped during handling these vials were discarded and no observations were recorded.

(c) Male competitive reproductive success

Male reproductive success of the evolved populations was measured by letting five males from the focal population compete with five *ebony* males for five *ebony* virgin females. After two days, the flies were discarded but the vials retained. All offspring that emerged from these vials were collected and the number of flies from each brood that were wild type or *ebony* was scored.

(d) Latency to copulation for naive males with a single receptive female

In order to determine whether flies from the evolved populations took relatively more or less time to mate with virgin, receptive females, individual males were placed into a standard vial with a receptive female in the afternoon, separated by a divider. The next morning, the dividers were removed and the latency to copulation scored for all males.

Flies that did not mate in 120 minutes were treated as right-censored observations in the analyses.

(e) Latency to copulation in the presence of unreceptive females

To measure the proficiency of individual males at mating with a virgin, receptive female in a complex social environment, groups of either 1 receptive and 1 unreceptive female or 1 receptive and 5 unreceptive females were shaken into vials containing 1 naive male. Latency to copulation was recorded. Flies that did not mate in 60 minutes were treated as right-censored observations in the subsequent analyses.

(f) Behavioral tracking in the presence of unreceptive females

As in the complex social environment assay outlined above, 5 unreceptive females and 1 receptive female where shaken into vials with individual males. Vials were scored every minute for 20 minutes for whether or not the male was courting and, if so, which class of female the male was courting. For males that successfully mated during the 20 minute window (16% in monogamy and 18% in polygamy, not significantly different between selection regimes), data was retained only for those minutes up to and including the onset of copulation.

(g) Olfactory learning

The olfactory learning paradigm [19] involves challenging flies to form an association between an odor (the conditioned stimulus, CS+) and an aversive mechanical shock (unconditioned stimulus, US). We measured the sexes independently by exposing same-sex groups of approximately 60 flies to three cycles of conditioning. In each cycle, flies were first exposed for 30 seconds to one odor (CS+) and subjected to shock (1 second of shaking every 5 seconds), followed by 60 seconds of air, another 30 seconds of the second odor (CS-) alone, and finally 60 more seconds of air. The two odors used in the learning assay were octanol and 4-methyl-cyclohexanol dissolved in paraffin (0.6 mL per L), each used equally as either CS+ or CS-.

One hour later, the flies were placed in a T-maze and allowed to choose between the two odors for 60 seconds. The number of flies in each arm of the T-maze was counted. Flies remaining in the central chamber were counted but did not differ numerically between selection regimes and were not included in the analysis.

Both odors used in the learning assay are known to be aversive to naïve flies. As a control for innate differences in how aversive the odors are to different populations, we also measured naïve flies in the T-maze.

(h) Activity levels

Two different measures of locomotor activity were obtained in order to test whether any differences between selection regimes in other assays might be attributable to activity levels. First, we used an assay to measure climbing response to shock described in [34]. Groups of twenty flies were tapped to the bottom of an apparatus consisting of two connected vials. The percentage of the flies that had climbed 8 centimeters within 10 seconds (the “climbing pass rate”) was recorded.

Next, we recorded the movements of individual males in transparent cylindrical chambers (1.2cm diameter x 0.8cm high) with webcams placed above the chamber. Males were first transferred to these chambers and allowed to recover for 10 minutes, then recorded for 5 minutes. We used the software CvMob (http://www.cvmob.ufba.br) to track the movement of the individual males and quantified activity as the total number of pixels traversed by the flies.

(i) Statistical analysis

All statistical analysis was performed in SAS 9.2 [35] using either PROC GLIMMIX for generalized linear mixed models (pseudo-likelihood estimation of parameters and Wald *F* tests for effect significance with degrees of freedoms computed by the containment method) or PROC NLMIXED for proportional hazard frailty models. Block effects were included in the linear mixed models as random effects when experiments were run across multiple days.

Competitive mating was analyzed with a generalized linear mixed model where the binomial response (offspring either wild type or *ebony*) was modeled with selection regime as a fixed effect and replicate population nested within selection regime as a random effect. Olfactory learning was modeled in the same way, separately for each sex, with the response variable the direction the fly moved in the T-maze (odor either correct or incorrect) and with the addition of odorant as a fixed effect. Following a convention [11, 36], we express learning performance as a learning score equal to $2P - 1$, where P is the proportion of flies choosing CS-.

The behavioral assays where latency to copulation was obtained for each male were analyzed using a time-to-failure/survival analysis framework. We used proportional hazards frailty models with an underlying log-logistic-distributed baseline hazard, accounting for right-censored data (males that never mated). Latency to copulation was modeled with selection regime as a fixed effect and replicate population nested with selection regime as a random (or 'frailty') effect. The assays involving 1 or 5 unreceptive females were modeled in the same way, with additional fixed effects for the number of unreceptive females present (1 or 5) and an interaction between selection regime and the number of unreceptive females.

The behavioral time series data were analyzed with a repeated measures generalized linear mixed model. Here, whether or not a male was courting (or whether he was courting the correct female) was a binomially-distributed response variable modeled with selection regime, time, and the selection regime x time interaction as fixed effects and replicate population nested within selection regime as a random effect. Because each fly was observed every minute for twenty minutes, the identity of each fly was included in the model as a random effect with a first-order autoregressive covariance structure (TYPE=AR(1) in SAS PROC GLIMMIX RANDOM statement) to account for the decay in covariance as distance between neighboring timepoints increases.

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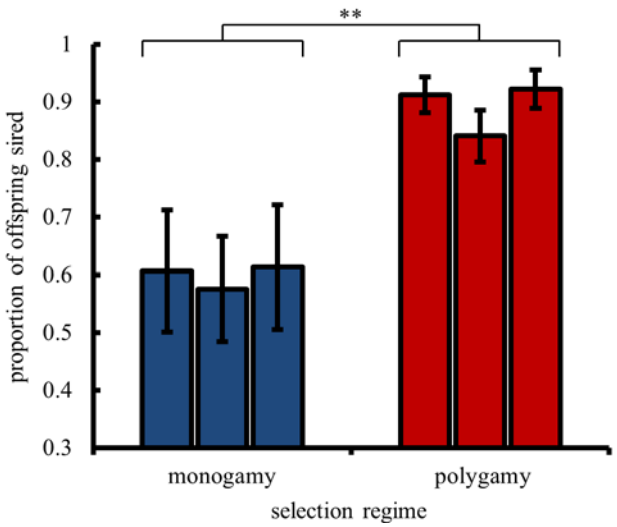

Figure 1. Competitive reproductive success for evolved males
The proportion of offspring (± S.E.) that was phenotypically wild-type, and therefore sired by males from the evolved populations, when males were placed in competition with *ebony* males for *ebony* females (n = 22 to 30 vials per population).

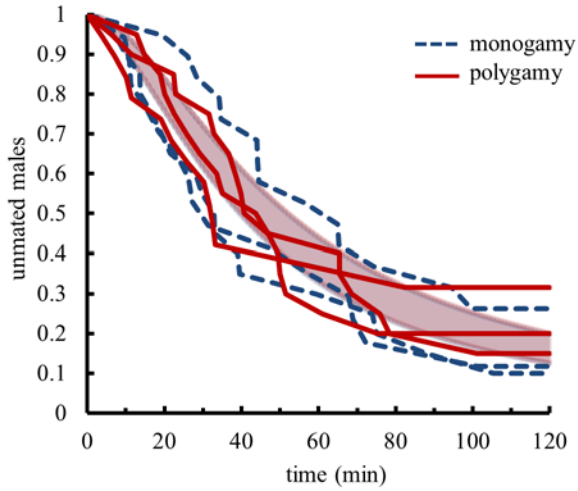

Figure 2. Latency to copulation for males placed with one virgin, receptive female
The proportion of males that have not mated over a two hour time course. Monogamous and polygamous populations are depicted in dashed blue and solid red, respectively, along with overlapping fitted curves and error bands (± S.E.) for each selection regime.

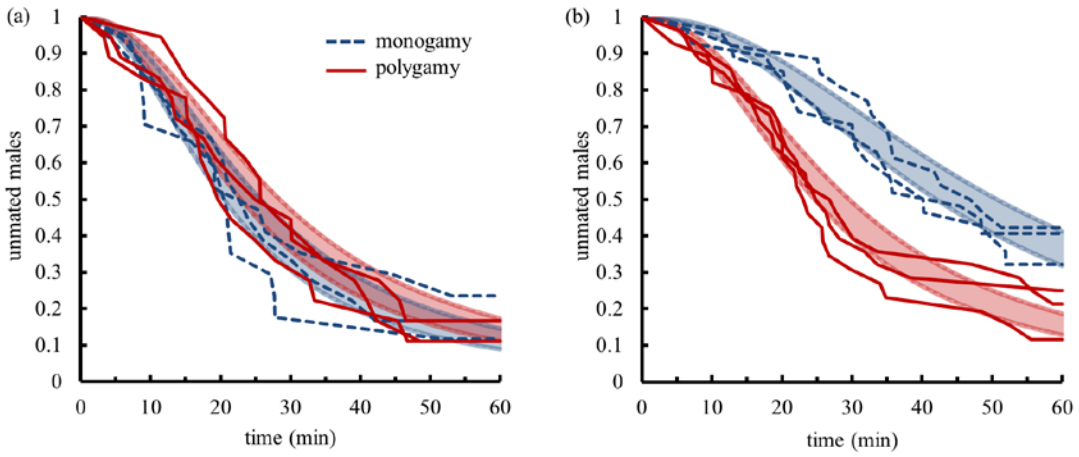

Figure 3. Latency to copulation for males faced with multiple females
The proportion of males that have not mated over a one hour time course when the environment consists of (A) one receptive and one unreceptive female (n = 17-18 males/population) or (B) one receptive and five unreceptive females (n = 26-28 males/population). The monogamous and polygamous populations are depicted in dashed blue and solid red, respectively, along with fitted curves and error bands (± S.E.) for each selection regime.

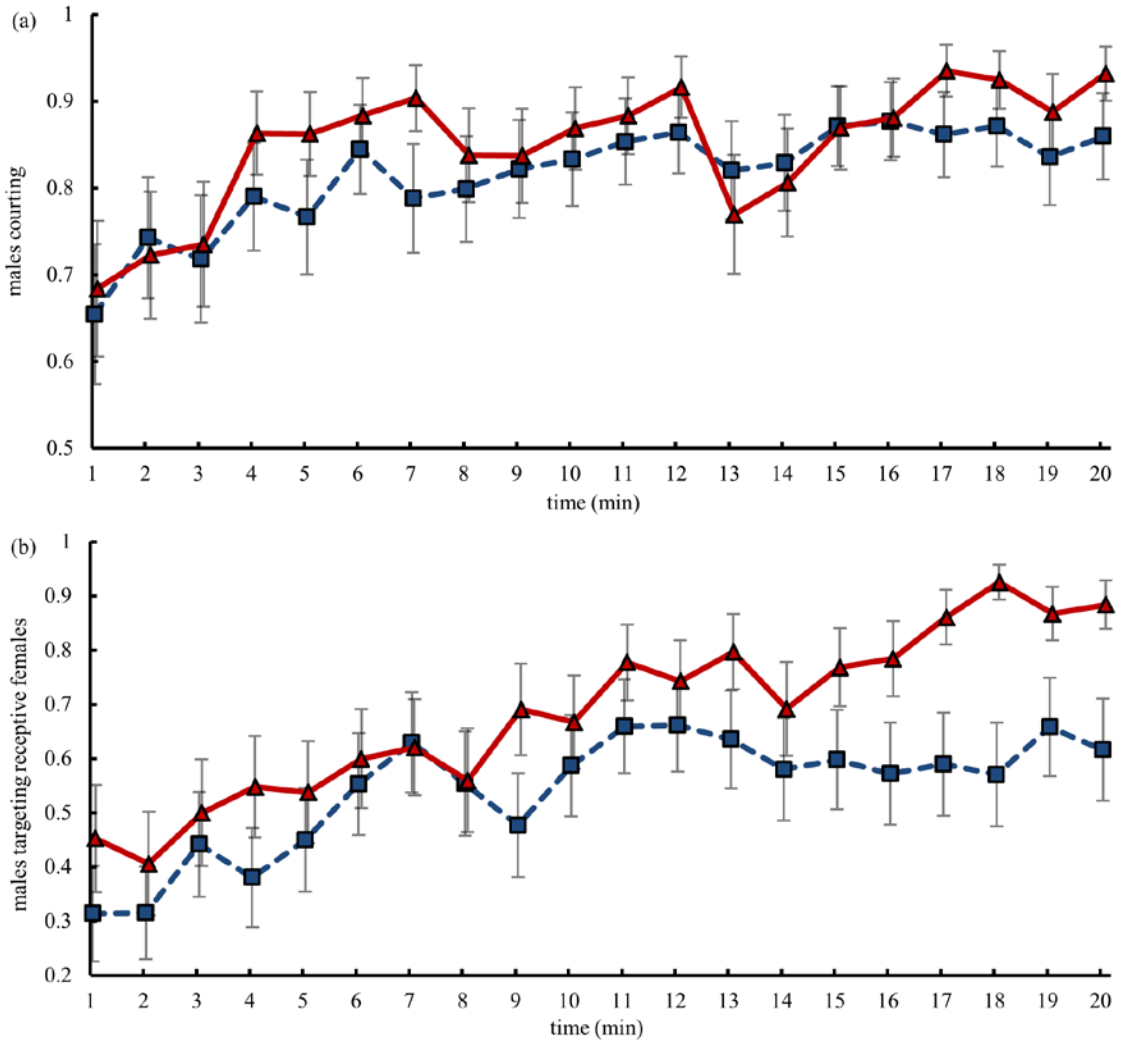

Figure 4. Courtship intensity and targeting
The proportion of males that are (A) actively courting and, if courting, (B) targeting the receptive female over a twenty minute time course when the environment consists of one receptive and five unreceptive females (n = 29-32 males/population). Means (± S.E.) are depicted for monogamous (blue squares) and polygamous (red triangles) selection regimes.

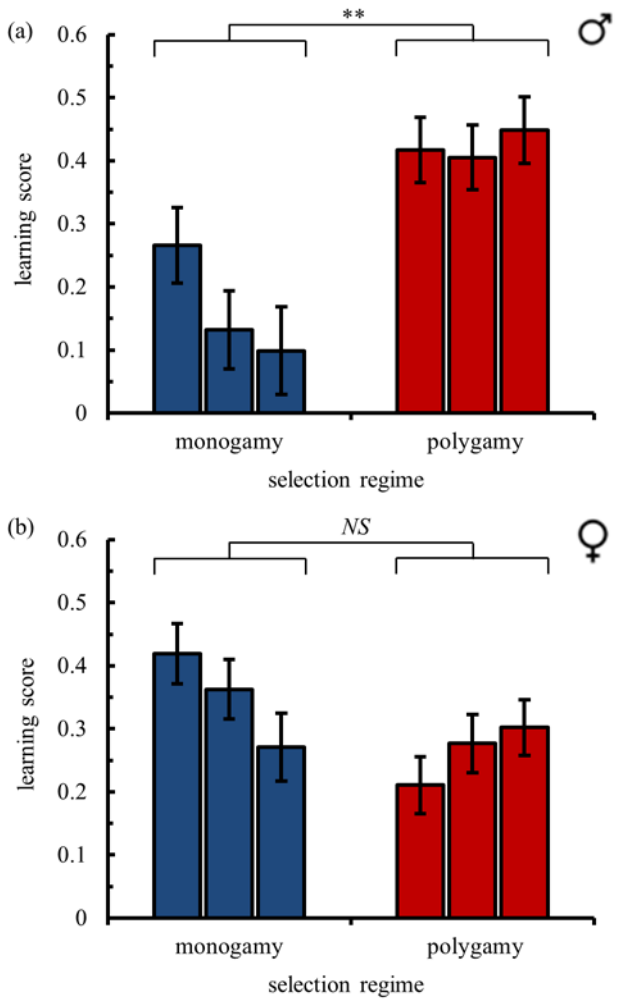

Figure 5. One-hour memory for evolved fly populations.
Learning scores (marginal mean ± S.E.) are shown for each population for (A) males and (B) females (n = 12 measures/sex/population).

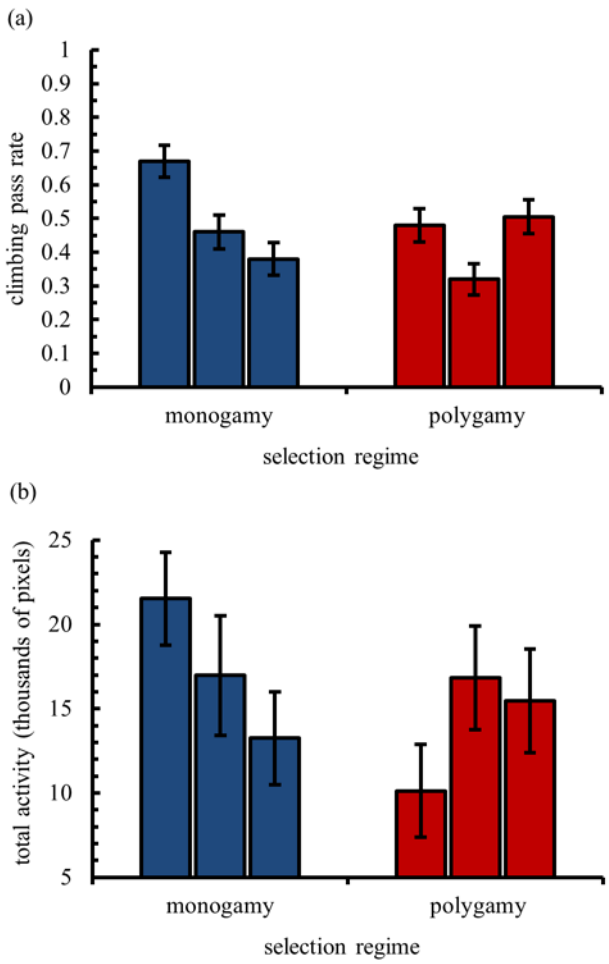

Figure S1. Activity levels
Activity levels as measured by (A) climbing response to shock (n = 5 measures/population) and (B) movement (n = 4-5 measures/population). In the climbing response to shock assay, the proportion of flies (± S.E.) passing the 8cm mark within 10 seconds is depicted for each population. In the movement assay, the mean number of pixels (± S.E.) traversed is depicted for each population.

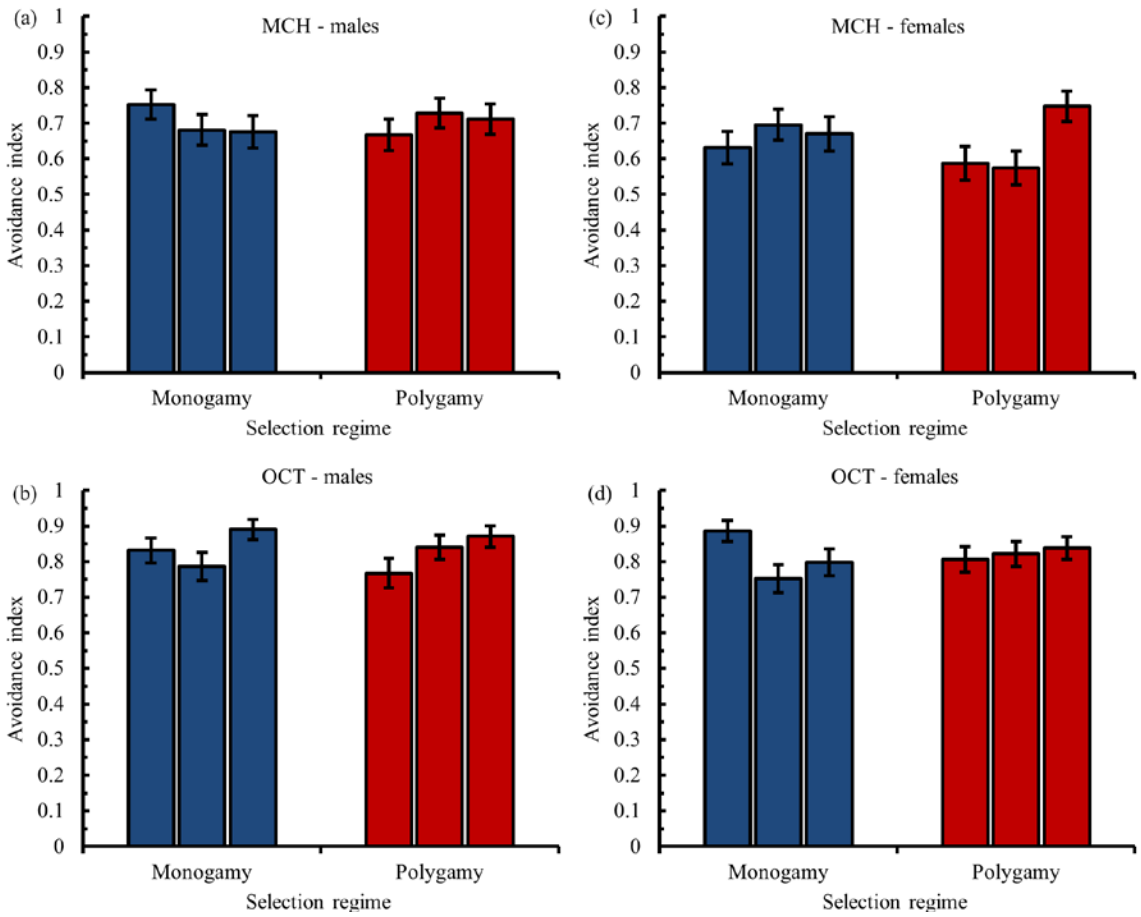

Figure S2. Innate odor aversion
The proportion of flies choosing solvent (octanol or methyl-cyclo-hexanol) over odorant for (A,B) males and (C,D) females (n = 3 measures/sex/population/odor). Naive males and females both avoid octanol more than methyl-cyclo-hexanol (males: $F_{1,29} = 31.52$, $p < .001$, females: $F_{1,29} = 48.20$, $p < .001$), but monogamous and polygamous flies do not differ in this innate avoidance (males: $F_{1,4} = 0.03$, $p = .86$, females: $F_{1,4} = 0.07$, $p = .81$).

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