

Hybridization and invasiveness in the freshwater snail *Melanooides tuberculata*: hybrid vigour is more important than increase in genetic variance

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Abstract

Many invasive taxa are hybrids, but how hybridization boosts the invasive process remains poorly known. We address this question in the clonal freshwater snail *Melanooides tuberculata* from Martinique, using three parental and two hybrid lines. We combine an extensive field survey (1990–2003) and a quantitative genetic experiment to show that hybrid lines have outcompeted their parents in natural habitats, and that this increased invasiveness co-occurred with pronounced shifts in life-history traits, such as growth, fecundity and juvenile size. Given the little time between hybrid creation and sampling, and the moderate standing genetic variance for life-history traits in hybrids, we show that some of the observed trait changes between parents and hybrids were unlikely to arise only by continuous selection. We therefore suggest that a large part of hybrid advantage stems from immediate heterosis upon hybridization.

Introduction

Most studies analysing the success of invasive species and their impact on indigenous taxa emphasize the ecological effects of invasive species on native ones, especially in relation to competition, predation and parasitism (Lodge, 1993; Williamson & Fitter, 1996). Evolutionary aspects of biological invasions have only recently received wider attention (Mooney & Cleland, 2001; Lee, 2002). Here we will focus on hybridization. Invasions may bring into contact related taxa which have been isolated for a long time (Huxel, 1999; Rahel, 2000; Pfenninger *et al.*, 2002), thereby creating opportunities for hybridization (Levin *et al.*, 1996; Allendorf *et al.*, 2001; Lamont *et al.*, 2003). Hybridization is increasingly recognized as potentially important in the

invasion process after introduction (Abbott, 1992; Levin *et al.*, 1996; Rhymer & Simberloff, 1996; Ellstrand & Schierenbeck, 2000; Sakai *et al.*, 2001). There is a growing list of recorded hybridizations between introduced species and native ones, mostly in plants (see Abbott, 1992; Ellstrand & Schierenbeck, 2000; Rieseberg *et al.*, 2003), and to a lesser extent in animals (Abernathy, 1994; Echelle & Echelle, 1997; Perry *et al.*, 2002). Hybridization between two invasive taxa has also been documented (Pysek *et al.*, 2003; Suehs *et al.*, 2004). In all these cases, hybrids outcompeted their parental taxa, leading to the reduction or extinction of one or both of them. Therefore, hybridization is now seen as a potential stimulus for the evolution of invasiveness (Ellstrand & Schierenbeck, 2000; Lee, 2002). Indeed, in contrast with the usual view of invasion as a purely destructive phenomenon for biodiversity, hybridization may generate evolutionary innovation (Anderson & Stebbins, 1954; Burke & Arnold, 2001; Seehausen, 2004) and may eventually create new species (Abbott, 1992; Baumel *et al.*, 2001). This raises the question of

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how hybrids can become competitively superior to their parental taxa and even more invasive.

Hybridization primarily affects the distribution of phenotypes, and two nonexclusive hypotheses can explain how this translates into hybrid success. Indeed, hybridization may change the mean and/or the variance of the phenotypic distribution (see Fig. 1a). Different effects are expected. (i) If the phenotypic mean changes (hypothesis 1), the average hybrid phenotype may lie outside the phenotypic range of its parents (Rieseberg *et al.*, 1999; Ellstrand & Schierenbeck, 2000; Perry *et al.*, 2001). With respect to fitness traits this phenomenon is known as heterosis (or hybrid vigour). Heterosis is expected when hybridization alleviates a high mutational load (Keller & Waller, 2002) or when there is some overdominance and/or cooperative epistasis between alleles inherited from the parental taxa. Changes in chromosome number, such as allopolyploidy, which frequently accompanies hybridization, may also contribute to the heterotic effect. However, some traits (e.g. growth) often show hybrid vigour whereas others (e.g. fecundity) may show outbreeding depression, resulting on the whole in lower fitness (Dobzhansky, 1970). Hybrids will be successful *in natura* only when the overall combination of traits increases fitness. (ii) The phenotypic variance can also change (hypothesis 2), as hybridization can generate new genotypes through recombination (Ellstrand & Schierenbeck, 2000; Bleeker, 2003). Introduced populations often have low genetic variance at neutral loci due to population bottlenecks, and might also have low levels of phenotypic variance,

which would limit their rate of adaptation to their new environment (but see Butlin & Tregenza, 1998; Reed & Frankham, 2001). Hybridization may thus alleviate the loss of genetic variance after founder events (Lee, 2002) and restore the efficiency of selection. This, of course, depends on the strength of the bottleneck.

Reproductive systems will strongly constrain which of these two mechanisms of hybrid superiority can operate. Reproductive systems stabilising hybridity (e.g. allopolyploidy or clonal reproduction) will maintain heterosis by fixing heterotic genotypes (Ellstrand & Schierenbeck, 2000), as they allow hybrids to avoid recombination and mixing with the parental taxa, which would generate suboptimal phenotypes. On the contrary, natural selection would be more efficient in sexually than in asexually reproducing hybrids because of the large number of new genetic combinations (in F2 and subsequent generations, as well as in backcrosses). The two processes can in theory be differentiated with respect to when and how evolutionary novelties are generated (see Fig. 1a). A change in phenotypic mean can immediately enhance fitness whereas fitness increase via a change in variance is postponed to later generations, once natural selection has had time to pick out the 'good combinations'. The dynamics of phenotypic changes is, however, rarely observed immediately after the first hybridization event in natural populations. Most likely, hybrids are detected when they are already abundant and have partially outcompeted their parental taxa: if original or favourable genetic combinations are found in hybrids, it is hard to know whether this results from heterosis or selection

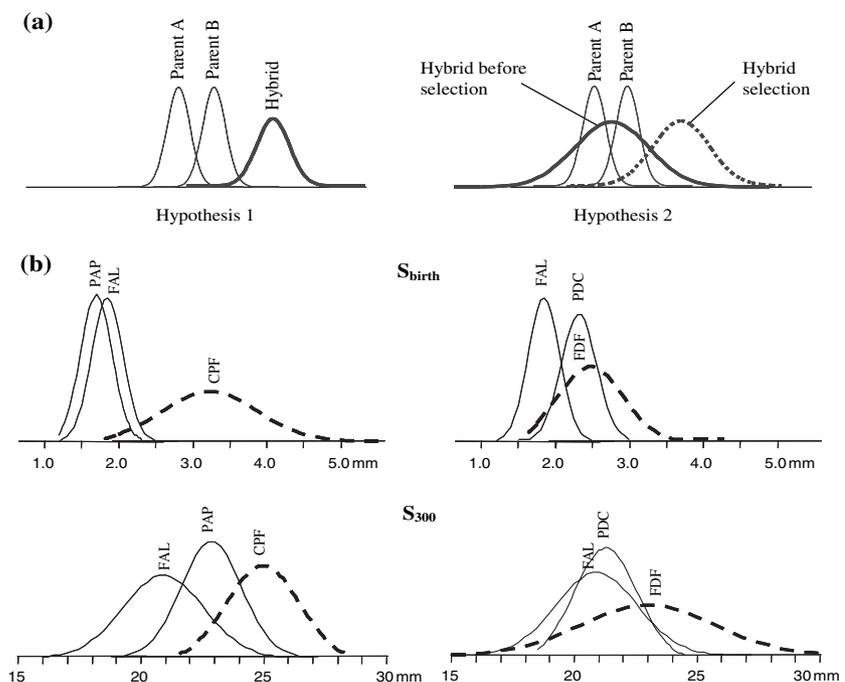


Fig. 1 (a) Theoretical frequency distributions of a normally-distributed trait for a hybrid and its parents according to the two hypotheses on hybrid fitness (see text). Hypothesis 1: the trait mean is increased following hybridization. Hypothesis 2: the trait variance is increased which is followed by a shift towards a higher mean value through natural selection. (b) Frequency distribution of two traits, size at birth (S_{birth}) and size at 300 days (S_{300}), for both hybrids (CPF and FDF on the left and right panels respectively) and their parents (FAL and PAP for CPF, and FAL and PDC for FDF).

within a hybrid swarm (hypothesis 1 vs. hypothesis 2 in Fig. 1a). It might also be difficult to test these hypotheses directly under laboratory conditions (but see Rieseberg *et al.*, 2003), because of technical difficulties (e.g. some species cannot be manipulated in the laboratory and/or it is not feasible to release potentially invasive artificial hybrids in order to evaluate their fitness in the field). An alternative solution to assess the amount and rate of selection response that would account for the observed difference is to sample hybrids in natural populations as early as possible after hybridization. If hybrids exhibit higher values than their parents, it is possible to infer the strength of selection required for producing such a pattern (given the number of generations since hybridization) which amounts to test hypothesis 2 (Fig. 1a).

We here evaluate the relative role of these mechanisms using, as a case study, the recent invasion of Martinique (French West Indies) freshwaters by the parthenogenetic Thiarid snail *Melanoïdes tuberculata*. Thanks to annual field surveys, the invasion sequence is well documented since the introduction of *M. tuberculata* in Martinique in 1979 (Pointier *et al.*, 1993; Pointier, 2001). Seven morphologically and genetically distinct morphs (or lines) of *M. tuberculata* have successfully established (Pointier, 2001; Facon *et al.*, 2003). Although Thiarids reproduce primarily by apomictic parthenogenesis, sexual reproduction occurs sporadically (Livshits *et al.*, 1984; Samadi *et al.*, 1999), giving rise to new parthenogenetic lines. Microsatellite data showed that two morphs, detected first in the early 1990s in Martinique, were hybrids between two pre-existing invasive morphs. Hybrid morphs are peculiar in several respects: First, they have an increased ploidy level compared with their parents, making them likely candidates for a heterotic effect. Secondly, unlike other morphs, each of them consists of several genetically distinct clones resulting from distinct primary hybridization events (see *Materials and methods* and Fig. 2). Assuming that hybrids display variation for quantitative traits as they do for molecular traits, this provides a basis for a possible action of natural selection after hybridization. So both heterosis (hypothesis 1) and selection (hypothesis 2) are plausible ways by which hybridization could alter the invasive performance of *M. tuberculata*, with unknown relative importance.

This natural context offers the opportunity to study the relationship between hybridization and invasiveness, because the origin of hybrids is both well documented and recent. We used a combination of field data and quantitative genetic experiments to answer the following questions. (i) Do hybrid morphs outcompete their parents in the invaded natural habitats? (ii) Do hybrid morphs differ genetically from their parents with respect to life-history and fitness traits? Can this explain their performance as invaders? (iii) Are the differences between hybrids and parental taxa consistent with an immediate heterotic effect of hybridization, and/or with

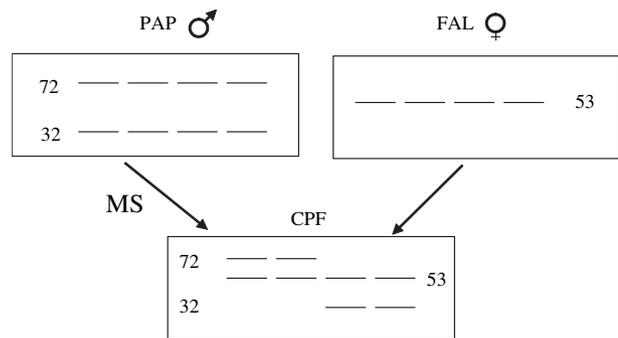


Fig. 2 The generation of a variable hybrid from invariable parents. The typical microsatellite patterns of CPF and its parents (PAP and FAL) are displayed (fragment lengths in pb). CPF is an aggregate of lines originated from distinct primary hybridisation events between PAP males and FAL females. Because of meiotic segregation (MS) during the production of male gametes, CPF lines have inherited various parts of the PAP genome. This pattern, represented here for locus 9, has been observed for two other loci that combine into a variety of multilocus genotypes. A similar process has been detected for FDF. Inspired from Samadi *et al.*, 1999.

an increase in genetic variance followed by natural selection (hypothesis 1 vs. 2)?

Materials and methods

The species studied and its invasion history in Martinique

Melanoïdes tuberculata is a tropical freshwater gastropod (Thiaridae) occupying permanent freshwaters (e.g. ponds, rivers) in tropical and subtropical environments. This viviparous species reproduces mainly through apomictic parthenogenesis (Jacob, 1959; Pointier *et al.*, 1993), although up to 40% males can be found in natural populations (Livshits *et al.*, 1984). Unambiguous evidence of sexual reproduction has been reported in Martinique only, and concerns the two hybrid morphs studied here. *Melanoïdes tuberculata* exhibits considerable variation in shell ornamentation which allows defining discrete morphs (Pointier, 1989; Pointier *et al.*, 1993, 2003). Microsatellite surveys have revealed that each morph corresponds to a genetically (and morphologically) homogeneous parthenogenetic line (Samadi *et al.*, 1999).

Before 1940, *M. tuberculata* was found in various regions of Asia and Africa. Since then, it has invaded the whole intertropical belt, mainly as a result of the trade of aquarium plants (Madsen & Frandsen, 1989; Glaubrecht, 2000). It was first mentioned in the New World (Texas) in 1964 (Murray, 1964), and its current distribution ranges from northern Argentina to Florida (Pointier, 1999; Quintana *et al.*, 2000). The New World was invaded by several morphs (Facon *et al.*, 2003). The

invasion process remains poorly known, except in the French West Indies where it has been thoroughly monitored, especially on the island of Martinique (Pointier *et al.*, 1993; Pointier, 2001). A regular survey was initiated in 1979, when *M. tuberculata* was first detected (Pointier, 1989). Seven morphs of *M. tuberculata* and two other Thiarid species (*Tarebia granifera* and *Melanoides amabilis*) have since then been observed.

We here focus on five morphs. Three of them (FAL, PAP and PDC; morphs are named using a three-letter code) arrived respectively in 1979, 1986 and 1991 (Pointier, 1989, 1999). They have distinct mitochondrial sequences and microsatellite patterns compared with other morphs in Martinique (Samadi *et al.*, 1999; Facon *et al.*, 2003). The two other morphs studied (CPF and FDF) are hybrids and were detected respectively in 1993 and 1995. Their hybrid status was first suspected based on morphological traits that appeared intermediate between those of the morphs mentioned above (PAP and FAL for CPF, and PDC and FAL for FDF). Microsatellite patterns indeed combine bands from the suspected parents (Samadi *et al.*, 1999). Identical mitochondrial haplotypes were identified in FAL, CPF and FDF, showing that FAL served as the maternal parent (Facon *et al.*, 2003). This is consistent with males having regularly been observed in PAP and PDC, though never in FAL (Samadi *et al.*, 1999). On the whole, the available data

suggest that hybridization involved unreduced female gametes from FAL and reduced (meiotic products) male gametes from PAP or PDC (Samadi *et al.*, 1999). Both hybrids therefore have an increased ploidy level compared with their parents, although the latter are already polyploid, as are all known parthenogenetic forms of *M. tuberculata* (Jacob, 1959, see also Baršienė *et al.*, 1996; Yaseen, 1996). Hybrids reproduce asexually and form new parthenogenetic lines.

As mentioned in the Introduction, no or very little nuclear and mitochondrial diversity has been detected within parental morphs (PAP, FAL and PDC), whereas both hybrids (CPF and FDF) exhibit microsatellite polymorphism (Samadi *et al.*, 1999; Facon *et al.*, 2003). Figure 2 describes how this polymorphism was generated by meiotic segregation in the heterozygous males.

Surveying snail populations in Martinique

The initial survey of natural populations of Thiarid snails in Martinique (1979–1993) included around 50 sites. It has since then been extended to 110 sites. We focus here on the 1990–2003 period and on sites in which hybrids were detected (Fig. 3). Sampling was performed manually (up to a few hundreds snails per site, when possible) and all Thiarids were collected randomly with respect to morph and species. Individuals were identified to the

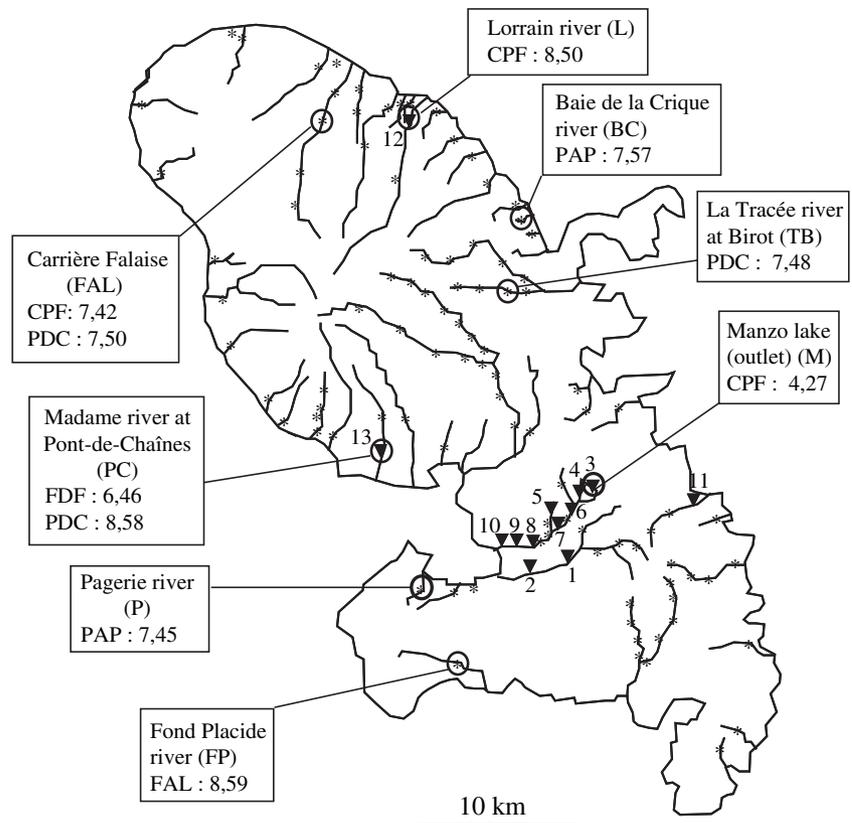


Fig. 3 Map of Martinique showing the sites studied during annual surveys of thiarid snails. Stars indicate sites in which hybrids were never observed, and solid triangles sites in which hybrids (CPF and FDF) were detected. Open circles indicate sites from which the individuals studied in the laboratory experiment were sampled. For each of these sites, the full and abbreviated names (in parentheses) are given, together with the identity of the morphs sampled together. The two numbers refer to the number of families and G_1 individuals studied. The population N of the FAL morph (eight families, 64 G_1 individuals) was sampled in Mare Nesmond (Marie-Galante), and is not depicted here.

species and morph levels. From this survey, we derived the number of sites occupied and the relative densities of each Thiarid line per site. At some sites, all morphs of *M. tuberculata* were rapidly displaced by another invader, *T. granifera*. Because this invasion considerably modified the environmental conditions and reduced the number of *Melanoides* to a few individuals per sample, such sites were excluded from the study as soon as *T. granifera* became dominant.

Measuring life-history traits in hybrids and their parents

Populations sampled, experimental design and rearing conditions

Two populations of both PAP and FAL were sampled in Martinique in December 2000 (Fig. 3). Given the decline of FAL in Martinique, one of the two populations of FAL was sampled in Marie Galante, another island of the French West Indies in which FAL arrived approximately at the same time as in Martinique. CPF and PDC were sampled in three populations and FDF in its only known population (Fig. 3). The populations were randomly chosen among those harbouring high snail densities of the relevant morph, except for FDF and FAL. It was not possible to sample parental and hybrid morphs in the same sites.

Seven adult individuals (G_0) per morph and population were isolated in the laboratory. Six to seven G_1 offspring per G_0 individual (a family) were isolated in 75 mL plastic boxes. Each box was filled with water originating from the Lez spring (near Montpellier) and fed *ad libitum* with boiled lettuce. Snails were maintained under a 12 : 12 L : D photoperiod. Water and food were changed once a week and the position of rearing boxes was randomized once a week. Because all G_1 offspring were collected over a month only, our protocol ensures that all G_1 snails were raised under standardized conditions throughout the experiment (1 year). The number of families and G_1 individuals studied is given per morph and population in Fig. 3. On the whole, the experiment included 77 families and 546 G_1 individuals.

Life-history traits

Several life-history traits related to reproduction and growth were measured in all G_1 individuals. These included the age (days) and size (from apex to the base of the spire, in mm) at first reproduction (A_{repro} and S_{repro} respectively). Size was recorded every 10 days during the first 3 months of life, and every month thereafter, using a microscope with a eyepiece micrometer. Growth curves were built using Richards model (Richards, 1959; Ebert, 1998) from which two parameters were retained, namely size at 300 days (S_{300}) which estimates the final size and the maximum instantaneous growth rate (V_{max}). The fecundity of G_1 individuals was quantified during the first 3 months of reproductive life, counting (and removing)

G_2 offspring every 3 days. From these data, we obtained individual fecundity curves, and estimated average daily fecundity (Fec). The size (S_{birth}) of nine G_2 individuals (0–3 days old) per G_1 parent was measured to the nearest 0.1 mm using the morphometric software Optimas 6.5©. To take into account temporal variation in birth size, the nine G_2 individuals were collected at three different dates (over about 2 months).

Statistical analyses

The following analyses were conducted for all life-history traits using the software JMP© (Sall & Lehman, 1996). Differences among morphs (M factor) were studied using analyses of variance (ANOVA). Data that did not conform to ANOVA assumptions were analysed after usual transformations. However, similar results were obtained and only results from untransformed data will be reported. Multiple-means comparisons were conducted on all traits both to test whether hybrids and their parents had different means, and to compare hybrid means to the arithmetic mean of parental means. Estimates of intra-morph components of variance were obtained from an ANOVA testing for both the population (P) and the family (F , nested within P) factors. Analyses were conducted for each morph separately because the intra-morph genetic variance was expected to differ among morphs. Population and family were entered as random effects. When nonsignificant, the F factor was discarded. The genetic variance was estimated as the sum of the variances corresponding to each factor ($V_G = V_P + V_F$). The broad sense heritability (H^2) was estimated as V_G/V_{tot} , with V_{tot} the total phenotypic variance. Note that H^2 differs from its usual meaning since it includes the population factor. The variances of life-history traits were compared between morphs using the genetic coefficient of variation (CV_G), which is the square root of the genetic variance (V_G) divided by trait mean (see Houle, 1992).

Assessing the plausibility of natural selection

Whether natural selection alone could be responsible for shifts in trait means between hybrids and their parents was determined as follows. Assuming that F_1 hybrids have the same phenotypic mean as the average of their parents (additive model), the breeder's equation gives the selection intensity needed for the trait to evolve to its current values by natural selection:

$$i = \frac{R}{H_p^2 \sigma_p}$$

where i is the intensity of selection, R the response to selection, H_p^2 the broad sense heritability within population (for an asexual species) and σ_p the square root of the total phenotypic variance. R was assumed to be equal to the difference between the observed means of hybrids and mean parental values, divided by the number of generations. We use the mid-parent value as a starting point to evaluate the plausibility of the null hypothesis

that selection alone is responsible from the observed trait shifts in *M. tuberculata* (no heterosis), and do not imply that hybrids should logically or generally be intermediate between their parental taxa. H_p^2 and σ_p were derived from the intra-population variance, because selection acts within a population. These quantities were assumed to have remained approximately constant since the first detection of hybrids in Martinique. Between the detection of CPF and FDF in Martinique (1993 and 1995 respectively) and the sampling date for this study (2000), the number of generations was 14–21 generations (CPF) and 10–15 generations (FDF), assuming 2–3 generations year⁻¹ in Martinique populations of *M. tuberculata* (Pointier *et al.*, 1992).

Results

Survey of snail populations in Martinique

The two hybrids exhibit different dynamics in the field (Fig. 4). CPF was first recorded in 1993 in the Coulisses

River at Petit-Fond, and then regularly increased to high frequencies relative to PAP, its paternal morph. During the few years following this first record, CPF was detected in another site from the same river (Coulisses River at Petit-Bourg) and invaded many sites of the neighbouring St-Pierre River. It was also detected in more distant river basins (Simon and Lorrain; Fig. 4). In all these sites, CPF tended to displace PAP. The maternal morph (FAL) was already declining before the appearance of CPF and it has now disappeared from all the sites where hybrids are present (Fig. 4). FAL was clearly outcompeted by PAP (or by other *M. tuberculata* morphs) before CPF occurred: the time window of PAP/FAL coexistence, allowing hybridization, was quite short in all sites, and where hybridization occurred (most probably Petit-Fond; Fig. 4), FAL became vanishingly rare before CPF reached detectable levels.

The other hybrid morph (FDF) has been detected in a single site (Madame River at Pont-de-Châines; Fig. 4). It was first recorded in 1995, and regularly increased in frequency relative to its paternal morph (PDC). As for

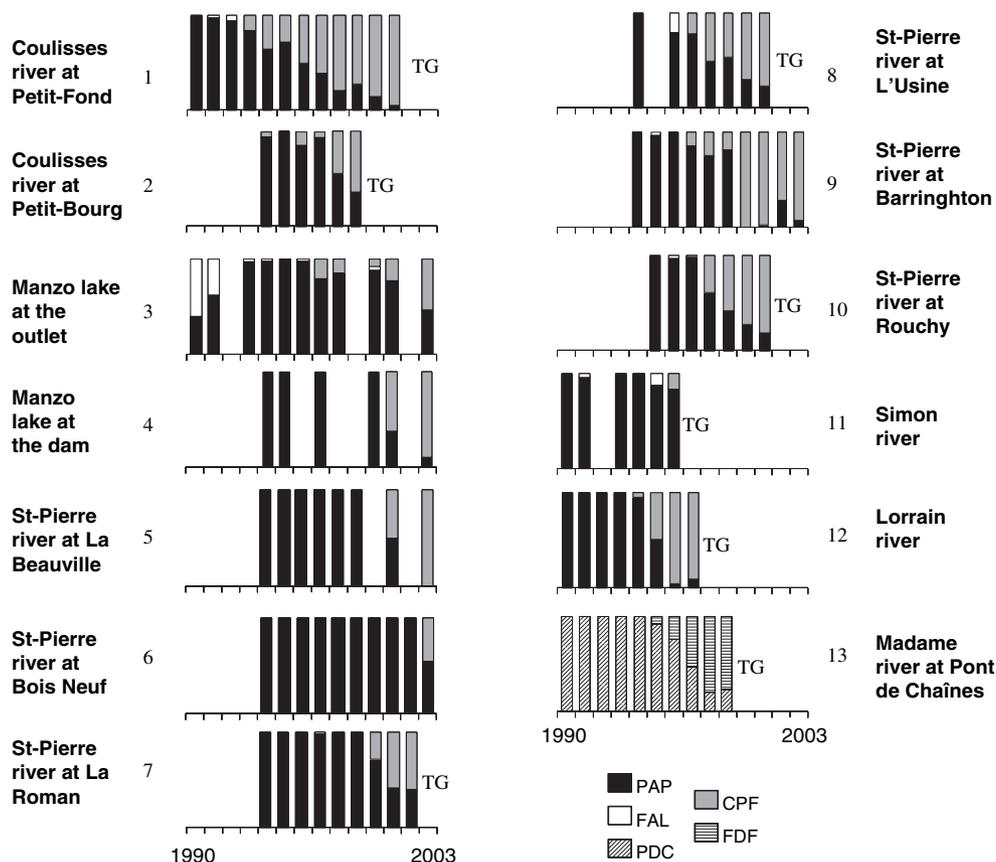


Fig. 4 Variation of the relative frequencies of hybrid morphs (CPF and FDF) of *Melanoides tuberculata* and their parents (FAL and PAP, FAL and PDC respectively) in the 13 sites from Martinique where hybrids were detected over the 1990–2003 period (see Fig. 2). Population numbers are those used in Fig. 2. In some sites (2, 4–10), the field survey was initiated in 1994. Missing data are because of too low densities or to local episodes of drought or flood. TG (on some panels) indicates that the site was fully invaded by *Tarebia granifera* (see *Materials and methods*).

CPF, the maternal morph (FAL) had disappeared before the FDF hybrid reached detectable numbers (Fig. 4).

Measuring life-history traits in hybrids and their parents

Mean values of the six life-history traits studied are reported in Fig. 5: highly significant differences were detected among morphs for all traits (ANOVAS, $P < 0.0001$; Table 1) especially for fecundity and size at birth. The percentage of variance explained by the Morph effect was relatively weak for A_{repro} (5%) and V_{max} (18%), but much higher for the other traits (55–74%; Table 1). For most traits, the parental morphs (FAL, PAP and PDC) displayed significantly different values. However the largest differences were generally observed between hybrids (CPF and FDF) and parental morphs. This holds whether hybrids exhibited higher (S_{300} and S_{birth}) or lower (Fec) means than the average of their respective parents (Fig. 5). For the maximum growth rate (V_{max}) and the size at first reproduction (S_{repro}), the CPF means exceeded the mid-parent values, whereas FDF was not significantly different from the mid-parent (Fig. 5). For A_{repro} , both hybrids were intermediate between their parents (Fig. 5), although the difference was small relative to trait means (<10% of trait means).

Estimates of genetic (population and family effects) and environmental components of variance are given in Table 2 for all morphs, and the corresponding CVs are reported in Fig. 6. The hybrid phenotypic variance was either equal to or higher (sometimes considerably so) than that of parents. Fecundity was the only exception with CPF showing less variance than PAP and FAL. However both the environmental and genetic CVs (variance scaled by mean) were higher for this trait in CPF than in its parents (Fig. 6). When the hybrid mean was much larger in hybrids than in their respective parents, an increase in phenotypic variance was also generally detected (see e.g. in Fig. 1b). However, this was sometimes mainly due to an increase in environmental rather than in genetic variance, and/or the contrast between hybrid and parents vanished when scaling by the mean (CV in Fig. 6). An increase in both the mean trait value and the genetic CV was detected for a single trait (S_{birth} in both CPF and FDF). In four cases, the genetic CV remained roughly constant despite the increase in mean trait value (S_{300} for both morphs and V_{max} and S_{repro} for CPF). In only one case (S_{repro} in FDF), an increased genetic CV was associated with a mean close to the midparent's.

The selection intensity required to explain the difference between hybrids and parental morphs was estima-

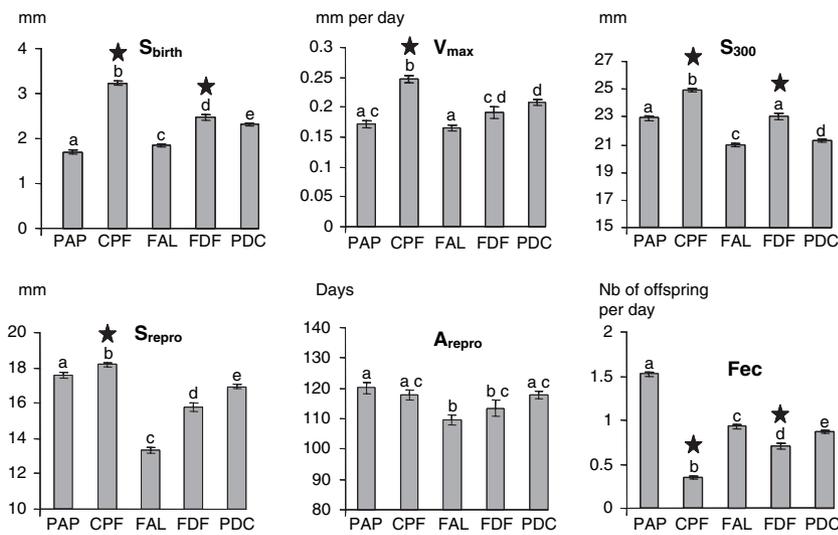


Fig. 5 Mean values and associated standard errors for each morph and life-history trait studied. Morphs sharing the same letter are not significantly different ($P > 0.05$). Stars indicate that the hybrid mean significantly differs from the average of its parents (CPF vs. PAP and FAL, FDF vs. PDC and FAL, $P < 0.05$). In each panel, hybrids are represented between their respective parents. The six life-history traits studied are: size at birth (S_{birth}), maximum instantaneous growth rate (V_{max}), size at 300 days (S_{300}), size and age at first reproduction (S_{repro} and A_{repro} respectively) and fecundity (Fec).

Trait	Mean	Morph SS (d.f. = 4)	Residual SS (d.f. = 541)	F-ratio	% Explained variance
S_{birth}	2.31 mm	166.60	74.56	302.53	74
V_{max}	0.20 mm day ⁻¹	0.50	2.69	24.91	18
S_{300}	22.44 mm	1268.12	1325.44	129.40	55
S_{repro}	16.41 mm	1726.40	1086.72	214.86	67
A_{repro}	116.02 days	7936.48	160442.21	6.69	5
Fec	0.87 offsp. day ⁻¹	77.58	40.38	259.85	71

Table 1 Means of the six traits studied and results of ANOVAS (test of the Morph effect). $P < 10^{-4}$ for all traits.

Table 2 Results of ANOVAS testing for the population (*P*) and family (*F*; nested in population) effects for each morph and trait. The variance component ($\text{Var} \times 100$ for S_{birth} and Fec and $\times 10^3$ for V_{max}) and its associated number of degrees of freedom (d.f.) and *P*-value (NS; $P > 0.05$), as well as the broad-sense heritability (H^2 ; see text for definition), are reported. Bold-type H^2 indicates that the whole model was significant. The population effect was not tested for FDF as a single population was studied.

Morph	Source	d.f.	S_{birth}			V_{max}			S_{300}			S_{repro}			A_{repro}			Fec		
			Var	<i>P</i>	H^2	Var	<i>P</i>	H^2	Var	<i>P</i>	H^2	Var	<i>P</i>	H^2	Var	<i>P</i>	H^2	Var	<i>P</i>	H^2
PAP	P	1	0.000	NS		0.017	NS		0.0000	NS		0.0171	NS		65.84	0.05		0.000	NS	
	F	12	0.361	NS	0.03	0.154	0.03		0.0000	NS		0.0000	NS		77.87	0.001		2.773	0.03	
	Res	88	4.090		0.046	1.074	0.137		1.5876		0.000	2.0792		0.000	265.22		0.351	19.029		0.098
CPF	P	2	9.379	0.01	0.003	1.437	0.003		0.7113	<0.001		0.3173	0.01		0.00	NS		0.000	NS	
	F	16	5.257	0.01		0.000	NS		0.0351	NS		0.0238	NS		44.42	0.02		0.655	<0.001	
	Res	100	28.795		0.337	11.126		1.7739		0.078	0.296	2.4213		0.123	287.97		0.100	1.795		0.229
FAL	P	1	0.087	NS		0.000	NS		0.1540	0.03		0.0000	NS		0.00	NS		0.220	NS	
	F	14	0.000	NS	0.01	0.498	0.01		0.1282	NS		0.3113	0.001		27.59	NS		0.243	NS	
	Res	107	4.465		0.013	3.032		2.4846		0.102	0.126	1.2642		0.170	297.23		0.074	3.936		0.105
FDF	F	5	3.834	0.02		0.301	NS		0.1643	NS		2.6590	<0.001		37.79	NS		0.035	NS	
	Res	40	14.748		0.206	3.914		7.1005		0.023	0.665	1.3389		0.067	258.33		0.128	6.071		0.006
	P	2	0.014	NS		0.000	NS		0.0645	NS		0.0000	NS		21.03	0.03		0.661	<0.001	
PDC	F	20	0.000	NS		0.000	NS		0.0000	NS		0.1131	NS		23.67	0.02		0.319	NS	
	Res	133	6.000		0.000	3.928		1.6700		0.008	0.008	1.3709		0.067	173.10		0.205	4.162		0.191

ted for each hybrid (CPF and FDF) for all traits showing significant hybrid advantage (see Fig. 5). Note that hybrid fecundity was lower than the parental ones: if this change was brought about by selection, it must be indirectly, through a negative genetic correlation with some positively selected trait. On the contrary, increases in S_{birth} , V_{max} , S_{300} , S_{repro} could be the result of direct directional selection. Assuming three or two generations per year, the selection intensity for CPF to evolve to its current birth size (S_{birth}) from the midparent value was 0.77–1.15 per generation. Assuming truncation selection and a normal distribution of birth size values, this amounts to removing 55–75% individuals per generation. For V_{max} , S_{300} and S_{repro} , all selection intensities exceeded 5, meaning more than 99.9% selective deaths per generation. This is because within-population heritability estimates were low and nonsignificant for these traits in CPF (0–0.02). Had the heritability for these traits been, say, 0.1, the numbers of selective deaths per generation would change to 20–33% (V_{max}), 66–86% (S_{300}), and 52–75% (S_{repro}). In FDF, the traits showing hybrid advantage were S_{birth} and S_{300} . The selection intensity was 0.29–0.43 for S_{birth} (selective truncation of 15–20% individuals) and 2.13–3.20 (95–99.5%) for S_{300} . Replacing the estimated nonsignificant heritability of 0.02 for S_{300} by 0.1, the latter became 0.48–0.72 (30–45% selective deaths).

Discussion

We will address successively the three questions asked at the end of *Introduction*.

Hybrid morphs outcompete their parents in natural habitats

Since their first occurrence (respectively 1993 for CPF and 1995 for FDF), the two hybrids studied regularly increased in frequency and are now largely dominant compared with their respective paternal morphs (PAP for CPF and PDC for FDF) in the sites where they were first detected. Comparison with the maternal morph (FAL) is more difficult, because FAL disappeared before the hybrids reached detectable numbers, being displaced by PAP or PDC (and in some sites also by other *M. tuberculata* morphs). However, assuming transitivity in ecological displacement relationships among morphs (if A is better than B, and B better than C, then A is better than C), both hybrids are expected to be able to outcompete their maternal morph. Note that all displacements among Thiarid lines observed so far in Martinique, either in the dataset presented here or in a larger dataset including all morphs and Thiarid species (data not shown), are consistent with the assumption of transitivity.

The performance of the two hybrids differed with regard to colonization of new sites. FDF has never been observed in other sites than the one in which it was first

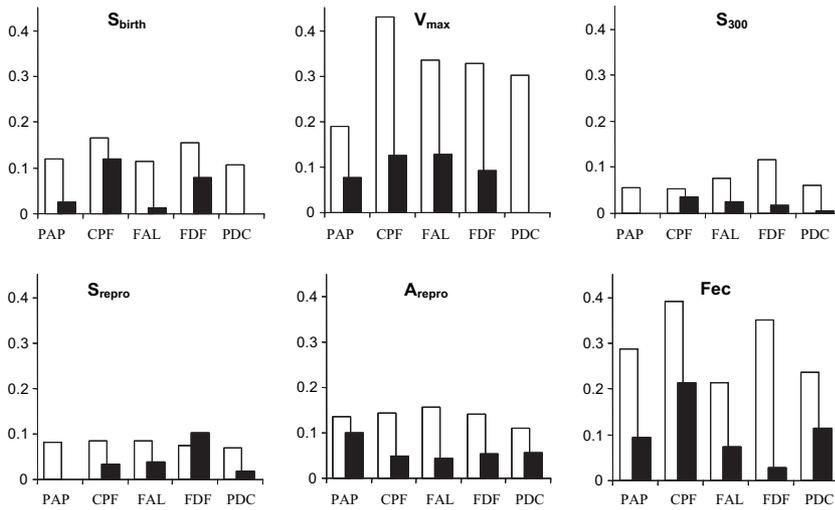


Fig. 6 Estimates of environmental (white bars) and genetic (black bars) coefficient of variation (CV; variance scaled by mean) for each morph and life-history trait studied. Within- and between-population components of genetic variance were pooled when estimating the genetic CV. Negative estimates were set to zero. The six life-history traits studied are: size at birth (S_{birth}), maximum instantaneous growth rate (V_{max}), size at 300 days (S_{300}), size and age at first reproduction (S_{repro} and A_{repro} respectively) and fecundity (Fec).

detected, including sites from the same river system (Madame River). On the contrary, CPF has colonized several sites both in the Coullisses River (where it first appeared) and in three other watersheds (Simon, Lorrain and Saint-Pierre river basins) during the 4 years following its first observation. It always outcompeted other morphs of *M. tuberculata*. The expansion of both hybrids has certainly been limited by contemporaneous invasions by *M. amabilis* and *T. granifera*. Both of these taxa very quickly invaded the Martinique freshwaters, reducing the fraction of available sites. *Tarebia granifera* appeared in most sites where CPF was present (Fig. 4) and quickly excluded most or all *M. tuberculata*. In 2003, *M. amabilis* and *T. granifera* had invaded more than 70% of the river basins from Martinique, and probably restricted invasion opportunities for CPF and other *M. tuberculata* morphs. In agreement with this hypothesis, a few individuals of CPF have been recovered occasionally from several rivers (not represented in Fig. 4) already colonized by large numbers of *T. granifera*, and the morph was not detected during the following years. Moreover no new watershed has been colonised by CPF after 1996. That FDF was apparently less successful than CPF may partly be due to its appearance in 1995, once *T. granifera* (first observed in 1991) was already dominant in many sites. However the inability of FDF to colonize upstream in the river where it first appeared, despite the lack of both *T. granifera* and *M. amabilis*, suggests that the FDF hybrid is not as performant as the CPF hybrid with regard to invasion dynamics.

Our study therefore indicates that the hybrids studied here display invasive superiority in natural populations, a pattern which has repeatedly been reported in plants (Thompson, 1991; Vila & D'Antonio, 1998; Bleeker, 2003; Figueroa *et al.*, 2003; Ayres *et al.*, 2004; see Abbott, 1992; Ellstrand & Schierenbeck, 2000 for reviews), but much more rarely in animals (see, e.g. Perry *et al.*, 2002

for exceptions). Whether increased invasiveness is a general attribute of hybrids remains open to further investigations.

Hybrid superiority in natura correlates with a genetic shift in life-history strategy

Hybridization giving birth to CPF and FDF had a strong impact on life-history traits and the shift is most pronounced for two traits. Hybrids displayed a much lower fecundity and a much larger size at birth than their parents. The largest contrast was found in CPF. The shift in mean value was of smaller magnitude for other traits, although significant for three traits in CPF and one trait in FDF. On the whole, hybridization in *M. tuberculata* has tended to shift life-histories towards larger investment into biomass per juvenile and growth, and lower number of juveniles. This shift upon hybridization mirrors that of invasive abilities *in natura*. Interestingly, CPF showed both a more pronounced life-history shift and a higher advantage in the field than FDF. Obviously low fecundity is not a factor of success *per se*. The hybrid advantage must therefore rely on greater investment per juvenile and in growth. This suggests that K-strategies proved more invasive than r-strategies in this context, an apparently counter-intuitive tendency that we will need to confirm and explain by further studies. Shifts in life-history traits associated with higher invasiveness have already been reported in some studies, especially in plants species. However, general patterns do not seem to emerge, as the traits concerned vary among taxa. For instance, Vila & D'Antonio (1998) attributed the superiority of *Carpobrotus* hybrids to an increased resistance to mammalian herbivory coupled with better clonal growth. In *Rhododendron*, a shift in cold-tolerance was shown to enhance invasiveness (Milne & Abbott, 2000). In the well-studied case of *Spartina*, vigorous growth

associated with greater output of pollen and seeds seemed to allow hybrid success (Ayres *et al.*, 2004). Although most studies focused on trait mean, they were not concerned with their variance.

Samadi *et al.* (1999) showed that each hybrid corresponds to several microsatellite genotypes, whereas the parental morph did not exhibit any intra-morph diversity. On this basis, we expected more genetic variance in hybrids than in parental morphs (PAP, FAL and PDC) for life-history traits. Moreover, recently introduced clonally-propagated parental strains are not expected to exhibit much variance. Our results suggest that it is not necessarily the case: several life-history traits exhibited nonzero genetic variance in the parental morphs at either the within-population, or the between-population level. Furthermore, the genetic variances were not systematically lower than those observed in hybrids (especially in FDF). Once environmental and scale effects are taken into account, hybrid genetic CVs clearly exceeded parental ones only for a minority of traits (birth size for both hybrids, size at first reproduction for FDF and fecundity in CPF). These results illustrate that patterns of genetic diversity at neutral markers may not necessarily predict variation at adaptive traits (see, e.g. Mc Kay & Latta, 2002).

Higher trait mean in hybrids is consistent with an immediate heterotic effect

The last question asked in *Introduction* (see also Fig. 1a) was whether hybridization simply affected levels of standing genetic variance at phenotypic traits, providing substrate for natural selection, or whether hybrids also benefited from an immediate increase in the mean of life-history traits. Let us focus on those traits that could experience direct positive selection (i.e. all but fecundity). Several distinct patterns were observed. First, for all traits except size at birth, increased mean (in at least one of the two hybrids) was not associated with increased genetic variance. Secondly, substantial increase in genetic variance was sometimes associated with no increase in mean value (size at first reproduction in FDF). Thirdly, size at birth exhibited both the largest difference in mean value between hybrids and parents and much more genetic variation in hybrids than in parents. On the whole, the most common pattern was 'increased mean/same variance'.

We interpret it as resulting from a direct heterotic effect (hypothesis 1; Fig. 1a). At least in CPF selection alone cannot account for the observed pattern. The reason is that extremely strong selection, especially in CPF (55–75% selective deaths per generation for size at birth, and more for other traits), would be required for the current trait values in hybrids to have evolved from a starting point near parental means through natural selection (hypothesis 2). The reproductive potential in hybrids is indeed unlikely to allow as much as 75% (or

more) juvenile mortality. First, hybrids displayed the lowest fecundities (and reproductive potential) of all *M. tuberculata* morphs studied here. A CPF individual produces on average between three and 21 juveniles per generation (assuming a generation time between 4 and 6 months) in laboratory competition-free conditions. Lower values are expected in natural populations. Secondly, a large proportion of juvenile mortality *in natura* probably results from stochastic demographic processes (e.g. flood). Thirdly, the hypothesis of truncation selection underestimates the number of selective deaths because it assumes maximal correlation between fitness and the selected trait. Natural selection does not usually proceed by truncation, and more deaths would be required to yield the same realized selection intensity. Fourthly, hybrids always rapidly reached very high densities after introduction (except when *T. granifera* invaded). As an example, the estimated density was 100–500 ind m⁻² in the Coulisses Petit-Fond site in 2000, 8 years after the first CPF individuals were observed. Such a rise only took 3 years in the Saint-Pierre River. Given the approximate size of the river basins, populations grew by a factor 100 per year (i.e. 4–10 per generation) during the years following first detection. This is hardly compatible with a very large selective load. Of course, it remains possible that our estimates of selection intensities are biased because constant heritability was assumed. For example, the current heritability of birth size in CPF could be lower than the initial one, precisely because of selection. However, most long-term artificial selection experiments show little decrease in heritability at the scale of a few tens of generations (e.g. Falconer & MacKay, 1996, pp. 216–217). Moreover the current heritability of birth size in CPF is by no means low (0.3), showing little sign of genetic erosion. It is tempting to draw a similar conclusion in FDF. However, lower selection intensities were detected for birth size, and this does not allow distinguishing between the two hypotheses. In fact, both life-history shifts and invasive success seem much less pronounced in FDF than in CPF.

The strong heterotic effect, observed at least in CPF, could result from an increase in ploidy level in hybrids. Several studies have highlighted that the combination 'hybridization/polyploidization' produces large shifts in life-history traits, including increases in cell volume, body size or seed/juvenile size, in both plants and invertebrates (Otto & Whitton, 2000). Such shifts have sometimes been associated with increased invasiveness, mostly in plants (Thompson, 1991; Soltis & Soltis, 2000; Bleeker, 2003). However, in all cases, it is admittedly difficult to separate the effects of hybridity *per se* from those of polyploidy (Otto & Whitton, 2000). For example, in predominantly asexual taxa such as *M. tuberculata*, both mechanisms may contribute to mask deleterious recessives accumulated through time within clonal lines. Furthermore, it is not possible to evaluate whether the phenotypic changes following hybridization and/or

polyploidy are always beneficial *in natura*. Only hybrids that are not outcompeted by their parents will eventually be detected. Such events are rare, and the generality of the heterotic effect hard to evaluate. Although creating hybrids under laboratory conditions would be difficult in *M. tuberculata* (at this point, we are not able to induce sex), studies on sunflowers show that this is a promising research direction for understanding the respective role of the mean and variance in life-history and fitness (Lexer *et al.*, 2003; Rieseberg *et al.*, 2003).

The impact of hybridization on invasion was immediately positive and mostly relied on heterosis, at least in CPF. Delayed effects due to the action of selection on increased genetic variance were probably limited. Because hybrids reproduce clonally, their genetic diversity cannot be regenerated through recombination (with themselves or their parents) after their creation. In nonclonal systems, it remains to be investigated whether recombination would generate enough genetic variance for significant evolution beyond what is allowed by immediate heterosis. This question could be addressed from quantitative genetic experiments, as shown here. Whatsoever our study is consistent with the idea that hybridization is an important evolutionary process, because of its ability to rapidly create evolutionary novelty (Anderson & Stebbins, 1954; Arnold & Hodges, 1995; Rieseberg *et al.*, 1999; Seehausen, 2004).

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