Frequency-dependent numerical dynamics in mosquitofish

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Altering the genetic composition of a population can alter several aspects of its numerical dynamics. Whether natural populations routinely contain the genetic variation capable of affecting the stability of those dynamics is less clear. Here we report a study of experimental populations of mosquitofish (*Gambusia holbrooki*), designed to examine this issue. The experiment examined the numerical effects of varying the initial relative frequency of a rare male genotype. A higher relative frequency of the rare, melanistic genotype produced higher mortality rates in melanistic males, higher mortality rates in females, higher juvenile abundance, and fewer fluctuations in the numbers of females across time. This work demonstrates that a natural population can harbour genetic variants in a single gender that are capable of inducing qualitative differences in the numerical dynamics of the opposite gender, through the effects of negative frequency-dependent selection.

Keywords: numerical dynamics; negative frequency-dependent selection; colour polymorphism; melanism; mosquitofish

1. INTRODUCTION

The numerical dynamics of populations is driven by exogenous forces that perturb densities and by endogenous properties that set birth and death rates as functions of density, and thereby govern rates of population growth and responses to perturbations of density (Ellner & Turchin 1995). The responses of birth and death rates to density determine the possible equilibria for population density and the stability properties in the neighbourhood of each equilibrium (Nisbet & Gurney 1982). In general, neighbourhood stability is enhanced by any factor that moderates the sensitivity of birth and death rates to variation in density, or that reduces the lag time between a change in density and a change in birth or death rates (exceptions exist in complex models: see Nisbet & Gurney (1982); figure 1). Considerable ecological research has focused on describing the levels of stability in natural populations, and on understanding the specific biological factors that create tendencies towards more stable dynamics (e.g. Hassell *et al.* 1976; Godfray *et al.* 1992; Turchin & Taylor 1992; Murdoch 1994).

A substantial theoretical literature has focused on whether the stability of population dynamics could be a by-product of Darwinian evolution (reviewed by Travis & Mueller 1989; Travis 1990). In these models, phenotypes (or genotypes in models with explicit genetics) vary in a trait, such as reproductive effort or competitive ability, that responds to density variation and that affects survival or reproductive output. In the joint dynamics of numbers and phenotypic (or genetic) variation, invading phenotypes (genotypes) that are less sensitive to the depressant effects of density on vital rates, or that respond more quickly to those effects, will often supplant resident types (Asmussen 1979; Mueller & Ayala 1981; Hansen 1992;

Doebeli & Koella 1995; figure 1). Enhanced numerical stability is an evolutionary by-product of this adaptive process.

In one variation on this theme, the fitnesses of different phenotypes (genotypes) lead to a protected polymorphism (because each can invade, but not drive the others extinct) and a stable equilibrium of genotype frequencies and total numbers. In these models, stability can be enhanced at the equilibrium when there are trade-offs among phenotypes (genotypes) in their absolute fitnesses near the equilibrium. These trade-offs allow population size and relative frequency of each phenotype (genotype) to move quickly and smoothly towards an equilibrium after a perturbation. Enhanced stability thus evolves again as a by-product of the adaptive evolution towards the equilibrium phenotypic (genotypic) configuration. A variety of models describe this idea with mechanisms ranging from various forms of contest competition (Lomnicki 1988; Lomnicki & Sedziwy 1989) to quantitative genetic variation in an ecological trait (Doebeli 1996) to frequency-dependent competitive interactions that create balanced polymorphisms (Asmussen 1983a,b; Clarke & Partridge 1988).

Our empirical knowledge about the evolution of population stability is less advanced. Laboratory systems offer long-standing evidence that altering the genetic composition of a population can alter one or more aspects of its numerical dynamics (e.g. Lewontin 1955; Park *et al.* 1964; Sokal & Karten 1964; Ayala 1966; Luckinbill 1978, 1979; Marks 1982; Mueller & Huynh 1994; Dennis *et al.* 1997), but, to our knowledge, only two studies have addressed the potential evolution of greater stability. In one, greater stability evolved as a by-product of the response to density-dependent selection (Mueller 1988). In the other, Stokes *et al.* (1988) examined the data from Nicholson's famous blowfly experiment and showed that the changing pattern of population dynamics during that experiment was consistent with changing demographic parameters that would provide greater stability.

It is unclear whether many natural populations typically

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Figure 1. Simplified model of birth (b) and death (d) rates plotted against population density in the neighbourhood of an equilibrium. The equilibrium density is at the intersection of the rates; the stability of the equilibrium is determined by the difference between the slopes of the vital rates on population density. Here, two phenotypes have different density-dependent death rates and density-dependent selection favours the phenotype with the higher equilibrium density, which in this case also confers a greater level of stability because of its shallower slope of death rate on density (see Nisbet & Gurney 1982).

harbour the genetic variation that would create distinct numerical dynamics and allow the latitude for stability to evolve (but see Sinervo *et al.* 2000). Genetic variation has been found for population parameters such as rate of population growth (Wade 1991), equilibrium population size (Dobzhansky & Spassky 1944; Birch *et al.* 1963), cannibalism (Lloyd 1968), and competitive ability (Bradshaw & Holzapfel 1989; see also Antonovics & Via 1987). To our knowledge, only three studies have attempted to detect the genetically based qualitative distinctions that would form the raw material for the evolution of stability. Leips *et al.* (2000) found additive genetic distinctions in population growth rates at low densities among conspecific populations of the least killifish, *Heterandria formosa*, but no distinctions in equilibrium population sizes or in responses of mortality and recruitment rates to density. Underwood & Rausher (2000) demonstrated that qualitative differences in herbivore dynamics were generated by genotypic variation among host plants. The only demonstrable link between allele frequency variation from a natural genetic polymorphism and numerical dynamics was reported by Sinervo *et al.* (2000). They found that a genetic polymorphism protected by frequency- and density-dependent selection drove long-term two-point cycles in numerical dynamics and allele frequencies in the side-blotched lizard, *Uta stansburiana*. Other links between genetic variation and numerical dynamics have been hypothesized (e.g. Chitty 1967) but not demonstrated.

Here, we report a study of experimental populations of mosquitofish, *Gambusia holbrooki*, designed to examine whether varying the initial relative frequency of a rare male genotype can induce quantitative and qualitative changes in numerical dynamics. This work demonstrates that a natural population can harbour genetic variants in one

gender that are capable of inducing qualitative differences in the numerical dynamics of the other gender.

2. MATERIAL AND METHODS

(a) *The male colour polymorphism in Gambusia holbrooki*

Mosquitofish (*G. holbrooki*) are common inhabitants of freshwater lakes and streams in the southeastern United States (Wooten *et al.* 1988; Jordan *et al.* 1998). Most males are silvery-grey in colour; mottled-black (melanic) males occur rarely. The black coloration of melanic males is caused by the deposition of melanin into macromelanophores (Regan 1961). The melanic pattern exhibits sex linkage and in some populations displays temperature-dependent penetrance (Angus 1989; Horth 2001). The earliest reports of melanic males indicate that they were the exceptions in collections of mosquitofish (e.g. Myers 1925). The current literature indicates that they remain rare throughout the species' range (Snelson *et al.* 1986; McPeck 1992; Taylor *et al.* 1996). We surveyed 54 populations throughout Florida annually from 1996 to 1999, collecting at least 60 males per survey in 44 of those populations, and found melanic males to be consistently rare. Their relative frequency ranged from 0 to 0.20 (mean 0.01 ± 0.02 s.e.m.). In populations where they were present ($n = 31$), their average relative frequency was 0.033 (± 0.006 s.e.m.). Populations harbouring melanic males were scattered throughout the entire range sampled.

(b) *Experimental design and execution*

The persistent rarity of melanic males led us initially to consider whether their abundance is limited by negative frequency-dependent selection. Accordingly, an experiment was designed to examine the effects of varying the initial relative frequency of melanic males on their survival, the initial survival rates of silver males and females, and the subsequent numerical dynamics of populations.

The experiment was run in 19 outdoor mesocosms (850 l cattle-watering tanks) that have proved suitable for multigenerational studies of small fishes (Leips *et al.* 2000), including mosquitofish (Scribner & Avise 1994). The three treatments were: (i) no melanic males (control, five replicate populations); (ii) low relative frequency (0.033) of melanic males (eight replicate populations, hereafter termed the 'low-frequency treatment'); and (iii) high relative frequency (0.083) of melanic males (six replicate populations, hereafter the 'high-frequency treatment').

Each replicate population began with 80 female and 60 male fish on the 8th March 1997. The fish were wild-caught adults taken from the St Marks National Wildlife Refuge, Wakulla County, Florida, USA. The sex ratio of the founding adults was purposefully set to equal that of the natural population from which they were drawn. We collected about 3000 fish and sampled this collection haphazardly until all 19 replicates were complete. The high- and low-frequency treatments spanned the observed average frequency of 0.04 found at St Marks over several censuses. Thus, the low-frequency treatment began with two melanic and 58 silver males, and the high-frequency treatment began with five melanic and 55 silver males. We used higher replication in the low-frequency treatment because the chance events that might affect the survival of the two melanic fish in each replicate could cause results from this treatment to be more variable.

Mesocosms were established at the high end of the range of natural *Gambusia* density, as calculated from throw-trap data

summarized in Leips & Travis (1999). We analysed density data from three of Leips & Travis's (1999) field sites for samples collected from March to November for 3 years. The mean number of *Gambusia* collected in throw-traps in the field was 91.9 ± 71.98 fish per 850 litres (the volume of one mesocosm). The range in the field was 27–371 fish per 850 litre volume (from throw-trap collections in which *Gambusia* was present). Our mesocosm means were 213 (control), 190 (low-frequency) and 237 (high-frequency) total fish per mesocosm.

We established mesocosms following the general procedures described by Leips *et al.* (2000). We filled mesocosms with natural vegetation and periodically added zooplankton from a local lake. The objective was to mimic the natural abiotic habitat as closely as possible, while excluding predators. Six weeks after the beginning of the experiment, and at six week intervals until November 1997, we conducted censuses of all replicates, counting all males, females, and juveniles in each mesocosm. Six censuses were conducted in total. New adults were present by the second census, at 12 weeks. These adults represent offspring produced by females that were gravid when collected in the field, or that were inseminated early in the experiment. Scribner (1993) found maturation times of 21.7 days for males and 40.5 days for females in the laboratory, and Haake & Dean (1983) analysed otolith data from Florida mosquitofish indicating that they mature in 8–10 weeks. Variation in population density affects maturation timing in some *Gambusia* species (Campton & Gall 1988; but see Busack & Gall 1983), as does food availability and temperature (Vondracek *et al.* 1988; Meffe 1992). Because reproduction occurred continuously throughout the spring, summer, and early autumn, we estimate that one to three overlapping generations elapsed during the course of the experiment. *Gambusia* are seasonal reproducers (Robinson & Buchanan 1988, p. 338) and by September, Krumholz (1948) observed almost no reproduction in field fish. Similarly, Scribner's (1993) experimental pool populations decreased markedly (–37.7%) in size over the winter.

(c) Statistical analysis

We analysed the survival of the founding adult females and silver males from the beginning of the experiment to the first census with one-way analyses of variance on the arcsine-transformed proportion of survivors. This analysis was possible because juveniles did not have time to mature in the first six weeks of this experiment, so adult numbers reflected only the survival of the founding adults. In subsequent censuses, recruitment of new adults precluded further analyses of the founders' survival. In the low frequency treatment, melanic males could exhibit only one of three survival-rate values in each replicate (0, 0.5, and 1), so we used randomization and bootstrap tests to determine whether the treatments affected melanic male survival. These two methods gave the same result, and we report only the randomization result.

Aside from the founding melanic males, we could not discern the colour genotypes of adult males because exposure to low temperatures is required for expression of melanism in this population (Horth 2001). Analyses of male numbers after the first census therefore address only the total number of males and not the frequency of the colour genotypes. Males were removed from the mesocosms in November and exposed to low temperature, which induces melanic expression. Melanic male frequencies among surviving males, determined after cold exposure, were about the same as the initial treatment frequencies. However, few fish remained at the end of the study

Table 1. Survival of the founding adults at the first census (sixth week of the experiment).

treatment (melanic male frequency)	female	silver male	melanic male
high (0.083)	0.45	0.45	0.47
low (0.033)	0.71	0.49	0.81
control (0.00)	0.69	0.44	—

in each mesocosm so the number of males within replicates of a treatment were combined to determine the frequencies of melanism for each treatment after cold exposure in the laboratory, and to compare these numbers with the initial melanic treatment frequencies.

We examined the numbers of juvenile, female, and male individuals with repeated-measures analyses of variance on log-transformed values in order to detect treatment and temporal effects over the course of the study. We took advantage of the temporal ordering of the response variables and the even spacing of the censuses and used orthogonal polynomial contrasts across the repeated measures to dissect the temporal effects into single degree of freedom contrasts for their linear, and nonlinear (quadratic, cubic, quartic, quintic) trends (Winer 1971). This procedure offers greater power for repeated measures testing when responses are ordered and patterned smoothly, and is more robust: it does not require the usual assumption in repeated measures analysis of compound symmetry in the error matrix. For the sake of brevity, we report only linear, quadratic and higher-order (the sum of all effects above quadratic) effects because linear and quadratic effects capture the differences among treatments in the shapes of their temporal patterns.

3. RESULTS

(a) Survival of founding adults

While the founding silver males survived equally well in all treatments, the survival of the founding females and melanic males varied among treatments (table 1). Females and melanic males had lower survival rates (by 0.25 and 0.34, respectively) in the high-frequency treatment than they did in the other treatments. Within the high-frequency treatment, females, silver males and melanic males exhibited comparable survival rates (*ca.* 0.45). However, in the low-frequency and control treatments, female survival was *ca.* 25% higher than the survival of silver males. Melanic males survived at higher rates in the low-frequency treatment than they did in the high-frequency treatment. Their low-frequency-treatment survival was also substantially higher than the rate at which silver males survived in this treatment.

Statistical analysis confirmed three patterns. First, treatments did not differ in survival of silver males ($F = 1.90$, d.f. = 2,16, $p = 0.83$). Second, survival of females in the high-frequency treatment was 25% lower than survival in either the low-frequency or the control treatments, which did not differ from each other ($F = 6.15$, d.f. = 2,16, $p = 0.01$). Third, the 34% lower survival of melanic males in the high-frequency treatment than in the low-frequency treatment was greater than expected by chance alone. A difference this large or larger was observed in only 0.2% of 1000 resamplings of the data.

Figure 2. Female abundance over time. (Solid line, high relative frequency (0.083) of melanic males; dashed line, low relative frequency (0.033) of melanic males; dotted line, control (no melanic males).)

Figure 3. Juvenile abundance over time. (Solid line, high relative frequency (0.083) of melanic males; dashed line, low relative frequency (0.033) of melanic males; dotted line, control (no melanic males).)

(b) Numerical dynamics

From the first census onwards, the three treatments were quite different in composition and in the numerical dynamics of females (figures 2–4). The high-frequency treatment, in which female numbers were lowest at the first census, continued to exhibit the lowest number of adult females throughout the experiment (figure 2). After the first census, female numbers in this treatment rose to *ca.* 56% of the initial number and persisted at that level throughout the experiment. In the low-frequency and control treatments, female numbers rose more rapidly after the first census. During the first half of the experiment, the numbers of females in these two treatments attained levels as much as 50% above those seen in the high-frequency treatment. In the second half of the experiment, female numbers declined in the low-frequency and control treatments. Juvenile numbers were similar among treatments at their first census, but at subsequent censuses were substantially higher in the high-frequency treatment, at times as much as twice the numbers in the other treatments (figure 3). Juvenile numbers were lower in all treatments at the final census, because of the maturation of juveniles in earlier censuses into adults, the cessation of reproduction by adult females in early autumn, and possibly seasonal mortality. In all treatments, the numbers of males increased sharply after the first census as a result of recruitment from the juvenile stage, and declined gradually thereafter (figure 4).

Statistical analyses of the data for males and juveniles confirmed these broad patterns. The numbers of males changed significantly over time, but in a parallel fashion

Figure 4. Male abundance over time. (Solid line, high relative frequency (0.083) of melanic males; dashed line, low relative frequency (0.033) of melanic males; dotted line, control (no melanic males).)

across treatments and without any overall distinction among treatments (table 2). The sharp increase and slow decline in all treatments generated a significant quadratic contrast in the analysis. The net increase in males between the first and sixth censuses produced the significant linear contrast in the analysis. The different treatments did not produce differences on either the course of this trajectory, or the absolute level at which it was traced.

The change in juvenile numbers over the course of the experiment was also significant (table 3). Like the pattern in males, the rise and decline of juvenile numbers produced a significant quadratic contrast in the analysis. Unlike the pattern in males, the number of juveniles was significantly higher in the high-frequency treatment. The lack of significance for any polynomial contrast for the interaction of time and treatment indicated that the high-frequency treatment traced the same temporal path as the other treatments, but at a higher level.

The analyses of female numbers revealed significant qualitative differences among treatments (table 4). The consistently lower abundance of females in the high-frequency treatment generated a significant main effect of treatment. The overall temporal variation in female numbers produced a very strong, significant quadratic contrast. However, in this case the treatment levels did not trace parallel trajectories of females, as indicated by the significant linear contrast from the treatment–time interaction. This interaction indicates that the temporal trajectories of female numbers are different in at least one of the treatment levels, necessitating the analysis of each level separately (Winer 1971).

The application of orthogonal polynomial contrasts to the female abundances in each treatment level revealed the qualitative differences in their trajectories. In the high-frequency treatment, female abundance showed no significant temporal variation at any order (linear, quadratic, etc.). In the low-frequency treatment, female abundance exhibited a significant linear trend (net decrease over time: $F = 6.15$, d.f. = 1,7, $p = 0.04$) and a highly significant quadratic trend (significant rise and subsequent decline: $F = 21.65$, d.f. = 1,7, $p < 0.005$). In the control treatment, female abundance exhibited a highly significant quadratic trend (significant rise and subsequent decline: $F = 30.78$, d.f. = 1,7, $p = 0.005$).

Table 2. Repeated-measures ANOVA for log male abundance.

source	d.f.	SS	F-ratio	p
between treatments				
treatment (melanic male frequency)	2	0.067	0.04	n.s.
error	16	12.360		
within treatments				
time	5	84.452		
linear effect	1	26.042	122.41	0.0001
quadratic effect	1	31.602	158.04	0.0001
higher-order effects	3	26.808	66.24	0.0001
treatment by time	10	0.518		
linear effect	2	0.008	0.019	n.s.
quadratic effect	2	0.069	0.172	n.s.
higher-order effects	6	0.441	0.540	n.s.
error	80	13.079		
linear error	16	3.404		
quadratic error	16	3.199		
higher-order error	48	6.477		

Table 3. Repeated-measures ANOVA for log juvenile abundance.

source	d.f.	SS	F-ratio	p
between treatments				
treatment (melanic male frequency)	2	6.072	3.60	0.05
error	16	13.485		
within treatments				
time	5	5.880		
linear effect	1	2.591	2.94	n.s.
quadratic effect	1	2.328	7.18	0.02
higher-order effects	3	0.961	2.73	n.s.
treatment by time	10	2.144		
linear effect	2	0.767	0.44	n.s.
quadratic effect	2	0.696	1.07	n.s.
higher-order effects	6	0.680	0.96	n.s.
error	80	24.940		
linear error	16	14.096		
quadratic error	16	5.192		
higher-order error	48	5.652		

(c) *Comparison of initial and final frequencies of melanism*

In the high-frequency treatment, 10 melanic and 108 silver males survived exposure to cold, which yielded a final relative frequency of melanic males of 0.08. The surviving numbers in the low-frequency treatment (six melanic and 140 silver males) resulted in a final melanic male frequency of 0.04. Neither of these frequencies differs from the initial frequencies (high frequency: $\chi^2 = 0.003$, $p = 0.99$; low frequency: $G = 0.016$, $p = 0.90$).

4. DISCUSSION

(a) *The effect of varying initial frequency of melanic males*

The major distinction among treatments was between the high-frequency treatment and the others. A higher initial relative frequency of melanic males decreased the survival rate of founding females, increased the number of

juveniles in the population substantially, and maintained a lower, statistically constant number of adult females over time. Total male numbers showed no effects, although the founding melanic males survived at a much lower rate when they began at relatively high frequency.

These effects indicate that the demography of the high-frequency treatment was different from that of the other treatments. The lower numbers of females in the high-frequency treatment and the absence of an effect on total male numbers made the adult sex ratio more even in the high-frequency treatment: given that the high-frequency treatment had substantially more juveniles and fewer adult females, either the survival rate of juveniles to adulthood or the survival rate of the females over time, or both, must have been substantially lower.

We suggest that our results were produced by density- and frequency-dependent behavioural differences between the two types of males. Several lines of evidence support this suggestion. In a laboratory experiment, at relatively

Table 4. Repeated-measures ANOVA for log female abundance.

source	d.f.	SS	F-ratio	p
between treatments				
treatment (melanic male frequency)	2	1.811	3.77	0.04
error	16	3.845		
within treatments				
time	5	1.520		
linear effect	1	0.144	0.90	n.s.
quadratic effect	1	1.200	22.53	0.001
higher-order effects	3	0.175	0.71	n.s.
treatment by time	10	1.659		
linear effect	2	1.210	3.79	0.04
quadratic effect	2	0.248	2.33	n.s.
higher-order effects	6	0.201	0.71	n.s.
error	80	7.339		
linear effect	16	2.555		
quadratic effect	16	0.852		
higher-order effects	48	3.931		

high densities, melanic males were more aggressive towards females than silver males, and more combative with other males (Martin 1977). In what may be a response to this aggression, females associate more with silver males than melanic ones (Nelson & Planes 1993; Taylor *et al.* 1996; M. A. McPeck, unpublished data; but see Martin (1975) and Karplus & Algon (1996)). The mean number of fishes in our mesocosms was higher on average than the mean found in nature (at three field sites (Leips & Travis 1999)), but our experimental means never exceeded the maximum found in nature. In other work, one of us (L.H.) has shown striking and consistent behavioural differences between silver and melanic males. Both density and frequency appear to play a role in governing the behavioural differences between the male colour morphs. These considerations indicate a variety of hypotheses about how density- and frequency-dependent behavioural interactions could produce these demographic patterns. For example, heightened aggression by melanic males towards one another and towards females could produce lower survival of females in the high-frequency treatment. The gonopodium (male mating structure) is barbed (Constantz 1989) and can tear the female during mating, and after repeated matings females are observed to be bloody (L. Horth, personal observation). Lower female density could have resulted in increased juvenile numbers through the relaxation of density-dependent stress on fecundity and/or on juvenile survival. Continued levels of aggression could regulate the subsequent recruitment rates of adult females and post-maturation survival. This type of regulation could produce the stability of numbers seen in the high-frequency treatment. Whether or not this is the mechanism operating, our results show that varying the composition of male genotypes affects the demography and numerical dynamics of females. And this effect must have been accomplished through the associated changes in intersexual interactions that follow from exchanging melanic males for silver ones. The relatively constant numbers of females in the high-frequency treatment indicates that the genotypic variation among males and its behavioural correlates offers the raw material

through which stability might evolve. The theoretical literature on the evolution of stability includes several treatments of behavioural properties. Doebeli (1995a) and Schoombie & Getz (1998) describe how phenotypes with different behavioural responses to population density could affect numerical dynamics (also see Ohgushi 1995).

Behavioural variation is certainly known to be an important factor in numerical dynamics. Sinervo *et al.* (2001) have demonstrated how density- and frequency-dependent selection on colour and behaviour generates cycles of numbers and phenotype frequencies in lizards. L.H. is currently investigating how the behavioural differences between melanic and silver males respond to changes in population composition; whether those effects give rise to the patterns observed in this study is not yet known.

(b) *The evolution of stability*

The work reported here demonstrates that natural populations of male mosquitofish harbour genotypic variation that is capable of altering female numerical dynamics qualitatively and of increasing population stability in the short term. The search for such natural genetic variants is not trivial. First, the existence of relevant genetic variation is not assured; although a few laboratory systems have presented genetic variation for qualitative effects on numerical dynamics, some rigorous studies have failed to find such variation (e.g. Luckinbill 1984). Second, certain types of genetic variation can produce the evolution of decreased stability as a by-product of density-dependent selection, for example when particular constraints exist among demographic parameters (Hansen 1992; Ferriere & Gatto 1993; Doebeli & Koella 1995; Ebenmann *et al.* 1996; Van Dooren & Metz 1998; Johst *et al.* 1999). Given the existing theory, it is important to learn just which types of genetic variation are found in natural populations and then to determine whether such variants can qualitatively alter numerical dynamics.

These results indicate that mosquitofish populations might exhibit steadier dynamics when there is a mixture of melanic and silver males. Many natural populations

harbour such a mixture, although melanic males are always rare when they do occur. In this sense, our results might best exemplify the class of models in which stability evolves as a by-product of the dynamics around a protected polymorphism. This interpretation must remain qualitative; the effect was observed at relative frequencies of melanics that are higher than those typically observed in natural populations.

Our experimental results were designed to examine endogenous effects on numerical dynamics generated by intra- and intersexual aggression. By using natural fish densities, vegetation and prey, and by establishing our mesocosms outdoors where populations are subjected to natural abiotic conditions, we intended to promote natural intraspecific dynamics. Because natural settings are confounded by other selective pressures, such as abiotic stress, predation, competition and parasitism, we cannot easily address the precise relationship between the relative frequency of melanic males and the level of stability in natural mosquitofish populations. The effects of variation in melanic male frequency would have to be examined against this range of other effects. An experimental approach would require several levels of initial relative frequency of melanic males, the passage of many generations, and either different externally imposed regimes of exogenous influences on numerical dynamics, or the simultaneous evaluation of the effects of a multitude of selective pressures. An observational approach would require long time-series data from a number of populations that show consistent differences in the relative frequencies of melanic males. The prediction from our results is that populations with higher relative frequencies of melanic males should exhibit stronger tendencies towards population regulation.

Additional studies of the frequency- and density-dependent nature of fitness in melanic males could generate refined estimates of fitness relationships of silver and melanic males. These estimates could be integrated into models for the joint evolution of allele frequencies and population parameters (Asmussen 1979, 1983a) to allow more precise predictions, which in turn might permit a more focused approach to the collection of time-series data. Ultimately, it would be of interest to discern whether mosquitofish populations are most stable numerically at the allelic equilibrium of this potentially balanced polymorphism, as well as to determine whether the type of numerical dynamics induced by each male colour morph plays a key role in determining their long-term fitness (Doebeli 1995b; Heino *et al.* 1998).

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.