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TESTING FOR EPISTASIS BETWEEN DELETERIOUS MUTATIONS IN A PARASITOID WASP

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Abstract.—Determining the way in which deleterious mutations interact to effect fitness is crucial to numerous areas in evolutionary biology. For example, if each additional mutation leads to a greater decrease in log fitness than the last, termed synergistic epistasis, then sex and recombination provide an advantage because they enable deleterious mutations to be eliminated more efficiently. However, there is a severe shortage of relevant empirical data, especially of the form that can help test mutational explanations for the widespread occurrence of sex. Here, we test for epistasis in the parasitic wasp *Nasonia vitripennis,* examining the fitness consequences of chemically induced deleterious mutations. We examine two components of fitness, both of which are thought to be important in natural populations of parasitic wasps: longevity and egg production. Our results show synergistic epistasis for longevity, but not for egg production.

Key words.—Fitness, longevity, recombination, sex, synergistic.

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Determining the way in which deleterious mutations interact to effect fitness is crucial to numerous areas in evolutionary biology (Kondrashov 1993). For example, one of the greatest problems for evolutionary biology is explaining the widespread occurrence of sexual reproduction and the associated process of recombination (Maynard Smith 1978; Bell 1982; Kondrashov 1993; Hurst and Peck 1996; Barton and Charlesworth 1998; West et al. 1999b; Otto and Lenormand 2002). A leading explanation for sex states that it is advantageous because it facilitates the removal of deleterious mutations (Kondrashov 1982, 1993; Charlesworth 1990). This hypothesis, the Mutational Deterministic (MD) hypothesis, states that the elimination of deleterious mutations provides an advantage to sex when each additional deleterious mutation leads to a greater decrease in fitness than the last (termed synergistic epistasis, Kondrashov 1982; Charlesworth 1990). Synergistic epistasis leads to linkage disequilibria that slows down the removal of deleterious mutations (Barton and Charlesworth 1998). Sex breaks up these linkage disequilibria and, given a sufficiently high mutation rate, this advantage can more than make up for the two-fold cost of sex (Kondrashov 1982; Charlesworth 1990). Many other theoretical studies rely on the assumption of synergistic epistasis (see Kondrashov 1993), but for simplicity we will focus our discussion on sex.

Despite its central importance, there is a lack of empirical data that allow us to test the importance of synergistic epistasis. This is particularly the case with regards to explaining sex. Although there has been a recent surge of elegant experiments testing for epistasis, these have not been ideal for testing the MD hypothesis for five reasons. First, most of the experiments have been carried out on effectively asexual species with low mutation rate (e.g. *E. coli,* Elena and Lenski 1997; *C. elegans,* Peters and Keightley 2000; yeast, Wloch et al. 2001). There is no need to explain sex in these species, and it has been suggested that the form of epistasis may vary with mutation rate or organism complexity (Falush 1998; Hurst and Smith 1998; Dall and Cuthill 1999; West et al. 1999c; de La Pena et al. 2000). This would be particularly important if variation in the extent of epistasis helped explain the distribution of sex across species/populations (Bell 1982; West et al. 1999b). Second, some experiments (Mukai 1969; de Visser et al. 1996, 1997a; Wloch et al. 2001) lacked proper controls (Keightley 1996; West et al. 1998). Third, the deleterious mutations examined have often been of large effect, including visible mutations, and/or mutations in the homozygous state (Peters and Keightley 2000; Whitlock and Bourguet 2000). The MD relies on deleterious mutations of small effect in the heterozygous state. Fourth, artificial mutagens have been applied directly to females, which can induce mutations in mitochondria (Peters 1999; Peters and Keightley 2000)that may have very different fitness consequences (Lynch et al. 1995). Fifth, the majority of fitness assays were not carried out under competitive conditions. The fitness consequences of deleterious mutations may only be fully realized when females are forced to compete for limiting resources (Kondrashov and Houle 1994; Peck and Waxman 2000).

Here, we attempt to rectify these problems with an experiment to test for epistasis in the parasitic wasp *Nasonia vitripennis.* This insect reproduces sexually and has a genome size similar to that of *Drosophila* (Rasch et al. 1975). Our experimental design involves using an artificial mutagen ethylmethane sulfonate (EMS) to create females with different mutation loads. We then test how log fitness varies with the inferred number of new mutations induced. It is convenient to work on log fitness because on this scale epistasis can be tested for by examining the shape of the relationship between log fitness and the number of deleterious mutations—independent (multiplicative) interactions would lead to a linear relationship, whereas synergistic epistasis would lead to a nonlinear relationship, with the slope declining more steeply as the number of deleterious mutations increases (Charlesworth 1990). In an attempt to rectify previous prob-

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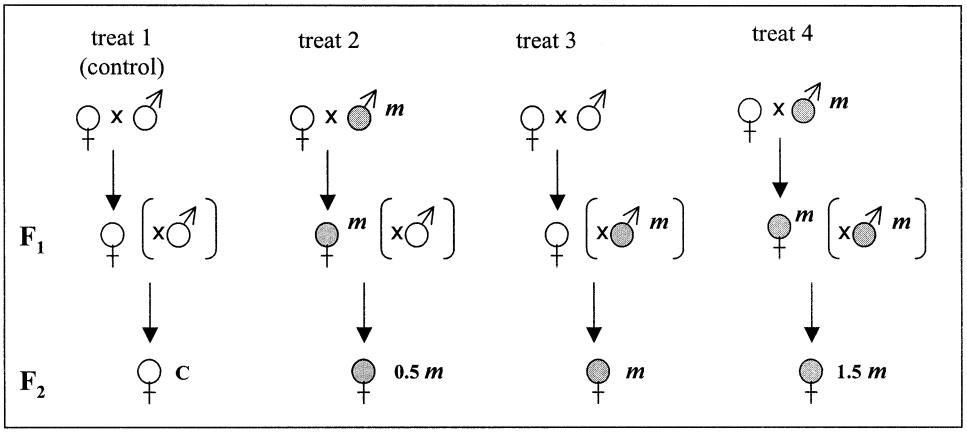

FIG. 1. Outline of the experimental design. Dark circles represent either treated males or females that have inherited mutations from their treated fathers or grandfathers (estimated relative number of mutations is represented by *m*). For each of the F_1 crossings, five F_2 offspring were selected to carry out the fitness measurements.

lems, we: (1) carry out mutagenis on males to avoid mutations in the mitochondria and to lose any mutations of large effect (males are haploid); (2) carry out crossings to assay the fitness consequences of new mutations in the heterozygous state; (3) use an eye color mutant to assay the fitness consequences of deleterious mutations in competitive conditions.

MATERIALS AND METHODS

The purpose of the experiment was to induce four different levels of deleterious mutations in *N. vitripennis* females and to quantify their effect on fitness. For this purpose, we used a chemical mutagen (EMS) thought to induce point mutations through the genome, especially G/C → A/T, at a rate dependent on the dose (Ashburner 1989; Anderson 1995; Keightley and Ohnishi 1998; Davies et al. 1999; Peters 1999). To avoid the potentially toxic effect of EMS on these females, the mutagen was applied either to their fathers, grandfathers, or both (see below). As this species is haplodiploid, fathers transfer all their genes to their female offspring, whereas mothers transfer on average half. Thus, the daughter of a male that has received *m* mutations will also have an average of *m* mutations, whereas the granddaughter will have 0.5 *m* mutations. A female whose father and grandfather were mutated will thus receive 1.5 *m* mutations (Fig. 1).

Nasonia vitripennis is a parasitoid wasp that attacks fly pupae (Whiting 1967). Experiments and laboratory cultures were carried out at 25°C (16:8 light:dark photoperiod) using pupae of *Calliphora vicina* (Diptera) as a host. Isofemale *N. vitripennis* lines, which originated from a stock of wild-type (black-eyed) wasps (LBII), kindly provided to us by L. Beukeboom (Institute of Ecological and Evolutionary Sciences, Leiden University). Lines were inbred by a series of father × daughter and brother × sister crosses until homozygosity was estimated to be > 99.9%. One of these lines (line 11) was selected at random to carry out the experiment.

Crossings

To obtain virgin males and females, the host can be opened ca. 12 days after parasitization, when the parasitoid pupae can be sexed (according to the size of the wing pads) and isolated individually in glass tubes (75 mm length, 10 mm diameter). Using this procedure, we isolated males and females at random. Twenty-four hours after emergence, half the males were treated with an 80 mM EMS solution (Sigma M0880) prepared in sugar water (10% w:v). One μl of this solution was placed on a 2 × 2 mm filter paper and placed inside the glass tube containing the male, which was then covered with a cotton stopper and left for 24 h for the EMS to act (treated males). The other half of the males were given the filter paper soaked in sugar water (untreated males). Males were then transferred to new tubes and each placed to mate with a single, newly emerged female for 24 h. A total of 50 treated-male x female and 50 untreated-male x female crosses were carried out in this way. Males were then removed and the females were given three large hosts for oviposition for a further 24 h. This protocol (large hosts, short oviposition time) was designed to limit the number of eggs laid and the competition between the developing larvae (to minimize selection). After this period, the female was discarded and the hosts placed at 25°C for the F_1 offspring to emerge. Shortly before emergence, one female parasitoid pupae from each cross was selected at random and isolated in a glass tube (as above). These F_1 females either carried *m* mutations inherited from the treated father (*m*-females), or no mutations (ø-females). Once emerged, half the females were crossed with a treated male (EMS dose and application as above) and half with an untreated male (following the same procedure as in the first crosses), so that four different treatment combinations were set up: ø-female × untreated male (n = 24), *m*-female × untreated male (n = 24), ø-female × treated male (n = X24), *m*-female × treated male (n = X23) (Fig. 1). Twenty-four hours later the males were discarded and the females given three hosts to oviposit on for a further 24 h. The hosts were then kept at 25°C for the offspring of the F_2 generation to develop. Twelve days later, five (F_2) female offspring pupae were selected at random from each male x female crossing, and placed individually in a labeled glass tube (tube measurements as above) in preparation for the fitness measurements.

TABLE 1. Deviance and *F*-values obtained for the linear and quadratic models and the difference between them. The proportion of the total deviance explained by each of the models is shown in parenthesis. Significant outliers have been eliminated from this analysis, but the results are qualitatively identical when they are included.

Fitness measurement	Linear model		Quadratic model		Difference	
	Deviance	*F*	Deviance	*F*	Deviance	*F*
Longevity	0.83 (7.7%)	$F_{1,94} = 7.80$**	1.27 (11.8%)	$F_{2,93} = 6.21$***	0.45	$F_{1,93} = 4.34$*
No. offspring	0.14 (1.5%)	$F_{1,92} = 1.44$	0.15 (1.7%)	$F_{2,91} = 0.78$	0.01	$F_{1,91} = 0.12$
No. offspring (+diap. larvae)	0.24 (2.1%)	$F_{1,92} = 1.97$	0.34 (3.0%)	$F_{2,91} = 1.41$	0.11	$F_{1,91} = 0.87$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Fitness Measurements

Twenty-four hours after emergence, each individual F_2 female was placed to mate with a newly emerged male taken from the stock line population for 24 h. The male was then discarded and the female provided with two hosts. In addition, to provide competition for the ovipositing female, a randomly chosen, mated red-eyed female (line Stdr, also provided by L. Beukeboom; eye color is recessive) was placed inside each tube. On every second day from this point on, the hosts were extracted and replaced by two randomly chosen new hosts until the death of the experimental female. To provide a constant competing pressure for the experimental female, if the red-eye female was found dead, it was immediately replaced by a new, randomly chosen one. All parasitized hosts were labeled and placed at 25°C for the offspring to emerge.

At the end of the experiment, the (black eyed) offspring produced by each experimental female were sexed and counted using a binocular microscope. To estimate the level of competition, the number of red-eyed offspring produced was also obtained. The hosts were opened and the number of unemerged diapausing larvae (Whiting 1967) counted, although it could not be determined whether they belonged to the experimental (black-eyed) or to the competing (red-eyed) female. Two different fitness measures for the F_2 females, longevity and offspring production, were obtained by averaging the five individuals selected from each crossing (see above). The relative importance of these two different measures is considered in the discussion.

TABLE 2. Deviance and *F*-values obtained for the factorial analysis of variance of the effect of the first and second mutagen doses. The full model includes the effect of the first and second doses plus their interaction. The significance of the interaction between the first and second dose can be interpreted as evidence of epistasis. Significant outliers have been eliminated from the analysis, but qualitatively similar results were obtained if they were included.

Fitness measurement	Full model		Interaction first × second dose	
	Deviance	*F*	Deviance	*F*
Longevity	1.36	$F_{3,92} = 4.40$**	0.44	$F_{1,92} = 4.29$*
No. offspring	0.17	$F_{3,90} = 0.58$	0.01	$F_{1,90} = 0.12$
No. offspring (+ diap larvae)	0.44	$F_{3,90} = 1.20$	0.11	$F_{1,90} = 0.87$

* $P < 0.005$, ** $P < 0.01$.

Statistical Analysis

Analysis was carried out using generalized linear modeling techniques available in the GLMStat Ver. 5.6.4 (Kagi Shareware, Ken Beath, Australia) statistical package. The analysis was carried out on the natural log of each of the two fitness measurements (longevity and offspring production). As we could not determine which female laid the diapausing larvae, offspring production was calculated in two different ways: (1) as the number of (black eyed) offspring emerged; and (2) as the number of (black-eyed) offspring emerged plus the number of diapausing larvae (i.e. assuming all diapausing larvae were laid by the experimental female). Grubbs tests (Sokal and Rohlf 1981) were carried out to detect statistically significant outliers. Three statistically significant outliers occurred in the analysis on offspring production (with and without diapausing larvae) and one for longevity—analyses with and without these outliers gave qualitatively identical results (both forms of analysis were carried out in case the outliers represented cases of mutations with large effects, in which we are not interested here).

To determine whether the mutations interacted epistatically, we carried out analysis in two ways. These ways are not independent, but both were used to test that our conclusions are not dependent on how analysis was carried out. First, we fitted a linear regression to the data ($\ln Y = c + \alpha m$, in which m = mutation dose, as described above). Whether the addition of the linear term caused a significant change in deviance was tested with an *F*-test. Then we fitted a quadratic term to the model ($\ln Y = c + \alpha m + \beta m^2$) and determined whether it significantly improved the accuracy of the prediction of the Y values. If the addition of this term resulted in a statistically significant change in the deviance of the model (Zar 1984), the quadratic equation was left in the model and the effect of mutation dose considered epistatic. The appropriateness of the final model, whether linear or polynomic, was tested by inspecting a plot of the residuals against the fitted values. Second, we carried out an analysis of variance (ANOVA) as done by Peters and Keightley (2000). We used a factorial design with two treatments: (1) grandfather mutagenized; and (2) father mutagenized. In this case, epistasis is tested for by examining the significance of the quadratic term. Model checking was carried out as with the regression.

RESULTS AND DISCUSSION

The results of the statistical analysis are summarized in Tables 1 and 2. Mutation dose has an epistatic effect on the

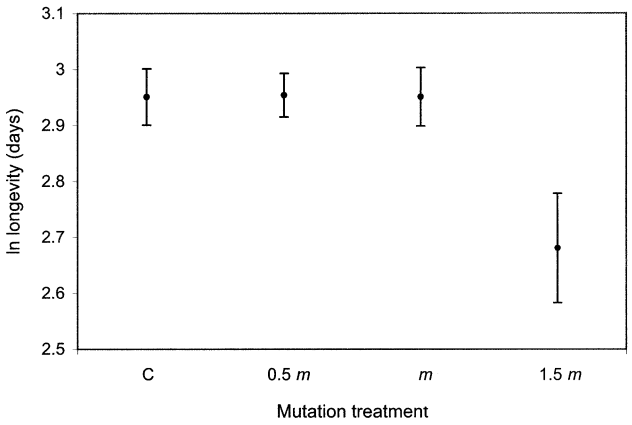

FIG. 2. Mean longevity (and standard errors) of the females allocated to each of the three mutation dose treatments. The one significant outlier (in the control treatment) has been eliminated from this graph.

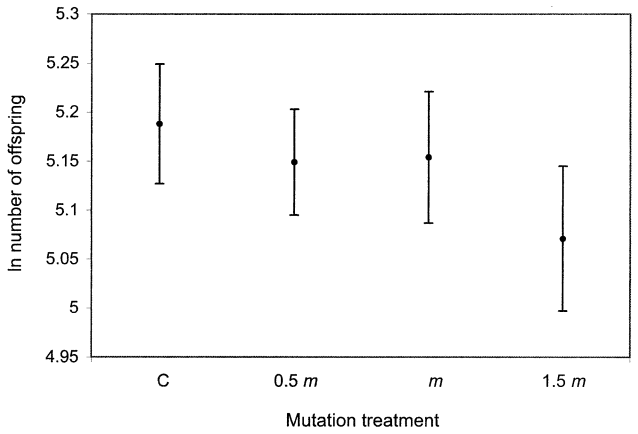

FIG. 3. Mean number of offspring (and standard errors) produced by the females allocated to each of the three mutation dose treatments. The three significant outliers (one in the control treatment and two in the 0.75 *m* treatment) have been eliminated.

longevity of the females (Fig. 2). The addition of the quadratic term to the model significantly increases the deviance of the model (significance of the quadratic term: $F_{1,93} = 4.34$, $P < 0.04$). The best-fit prediction of the longevity of females with $\times$ mutations is given by ln(longevity) = $2.75 + 0.26\ m - 0.07\ m^2$ (95% confidence intervals of the linear and quadratic terms: $\alpha = -0.07 \rightarrow 0.59$, $\beta = -0.13 \rightarrow -0.01$). Although the number of offspring seems to follow a similar trend (Fig. 3), neither the linear nor quadratic effects of mutation dose have a significant effect on offspring production. The number of diapausing larvae was significantly smaller in females allocated to the highest mutation dose treatment (1.5 *m*; $F_{3,92} = 3.89$, $P < 0.05$). Adding the diapausing larvae to the total number of emerged black-eyed offspring also rendered a nonsignificant effect of mutation dose (Table 1). The same conclusions were reached when analysis was carried out by ANOVA (Table 2). Keeping the outliers in the database did not change the significance of the results. The quadratic effect of mutation dose still had a statistically significant effect on longevity (Regression: $F_{1,94} = 5.44$, $P < 0.05$; ANOVA: $F_{1,93} = 5.38$, $P < 0.05$), but not on offspring production (Regression: $F_{1,94} = 3.09$, ns; ANOVA: $F_{1,93} = 3.06$, ns). When the diapausing larvae are added to the total number of offspring, the effects are marginally significant (Regression: $F_{1,94} = 3.87$, $P = 0.05$; ANOVA: $F_{1,93} = 3.82$, $P = 0.05$).

Our results suggest that in *N. vitripennis*, deleterious mutations show synergistic epistasis effects on longevity, but not on offspring production. The overall fitness consequences will therefore depend upon the relative importance in nature of these two fitness components. These lay at opposite ends of the continuum on what limits reproduction: longevity, the time available to find hosts (host- or time-limitation), or egg production, the number of eggs available to be laid once a host is found (egg-limitation). The relative importance of these two fitness components in parasitoids has been a subject of much debate. Recently, a consensus seems to have been reached, from both theoretical and empirical data, that species are generally at an intermediate position on the egg/host limitation continuum (Driessen and Hemerik 1992; Rosenheim 1996, 1999; Ellers et al. 1998, 2000; Sevenster et al. 1998; West et al. 1999a; Casas et al. 2000; West and Rivero 2000). This means that in parasitoids, fitness is influenced by both longevity and egg production, their relative importance depending on the characteristics of the environment in which the females forage.

Like other experiments that look at the fitness consequences of mutation accumulation, ours has limitations. Perhaps most importantly is the possibility that some mutations are lost during the accumulation process, and that these would have been most likely to be epistastic (de Visser et al. 1997b; Peters and Keightley 2000). We attempted to reduce this possibility by minimizing within host competition (small broods, large hosts) and sib mating (see Methods). However, the potential for this problem is shown by the fact that it was harder to obtain replicates per mutated line in the treatment with the highest mutation load (4.24 $\pm$ 0.28 SE, compared with 5.0 $\pm$ 0 in the other lines; see also Whitlock and Bourguet 2000). Consequently, our estimates must be taken as lower bounds on the possible importance of synergistic epistasis. One way to avoid this problem is to carry out experiments that control the mutational insertions, although this can limit the types of mutations that can be used (Elena and Lenski 1997; Whitlock and Bourguet 2000). Another limitation is that we have only measured components of fitness, albeit highly important ones in competitive circumstances.

We conclude with two general points. First, is there variation across species in the amount of epistasis, and if so, can we explain it? Deleterious mutation rates vary widely across species (Keightley and Eyre-Walker 2000), and arguments could be made that the amount of epistasis should vary with factors such as mutation rates, complexity, and life history (Falush 1998; Hurst and Smith 1998; Dall and Cuthill 1999; West et al. 1999c). However, although we know that epistasis can evolve (Malmberg 1977), this problem has lacked theoretical attention, and there are too few empirical data to address it—even before addressing the problem for comparison that arises when some estimates of the β/α ratio are negative, as found by Whitlock and Bourgeout 2000 and this study. Second, very small amounts of epistasis can be

important in explaining sex. In particular, the amount of epistasis required to explain sex can be reduced by: (1) spatial population structure, in which geographically closer individuals are more likely to mate and compete with each other (Brookfield 1999; Peck et al. 1999; Balloux and West, unpubl. ms); (2) sexual selection (Agrawal 2001; Siller 2001); (3) other mechanisms such as host-parasite coevolution simultaneously providing an advantage to sex (Howard and Lively 1994, 1998; West et al. 1999b). Within the context of *N. vitripennis*, we know that (1) is likely to be important; and (2) unimportant (Whiting 1967; Werren 1983; Molbo and Parker 1996).

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