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# Does reproductive isolation evolve faster in larger populations via sexually antagonistic coevolution?

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**Sexual conflict over reproductive investment can lead to sexually antagonistic coevolution and reproductive isolation. It has been suggested that, unlike most models of allopatric speciation, the evolution of reproductive isolation through sexually antagonistic coevolution will occur faster in large populations as these harbour greater levels of standing genetic variation, receive larger numbers of mutations and experience more intense sexual selection. We tested this in bruchid beetle populations (*Callosobruchus maculatus*) by manipulating population size and standing genetic variability in replicated lines derived from founders that had been released from sexual conflict for 90 generations. We found that after 19 generations of reintroduced sexual conflict, none of our treatments had evolved significant overall reproductive isolation among replicate lines. However, as predicted, measures of reproductive isolation tended to be greater among larger populations. We discuss our methodology, arguing that reproductive isolation is best examined by performing a matrix of allopatric and sympatric crosses whereas measurement of divergence requires crosses with a tester line.**

**Keywords:** sexual conflict; population size; experimental evolution; reproductive isolation

## 1. INTRODUCTION

Sexual conflict occurs when there is a conflict between the evolutionary interests of two mating partners (Parker 1979) and can lead to sexually antagonistic coevolution between males and females and rapid evolution of reproductive traits within populations (Holland & Rice 1998). This can result in a rapid evolution of reproductive isolation as a by-product of genetic divergence between allopatric populations. As a result, sexual conflict could be an engine for speciation (Rice & Holland 1997; Rice 1998). This hypothesis is supported by theory (Parker & Partridge

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1998; Gavrillets 2000), comparative analyses (Arnqvist *et al.* 2000) and experimental data (Martin & Hosken 2003). Unlike other models of allopatric speciation (e.g. Lande 1981; Gavrillets 1999), models of reproductive isolation by sexual conflict predict that large populations should evolve faster, because they harbour greater levels of standing genetic variation and have proportionally larger numbers of mutations to fuel evolutionary change, and because they experience more intense sexual selection due to larger census size (Gavrillets 2000; Tregenza 2003). However, while there have been experimental tests of some of these ideas, there have been no attempts to disentangle the effects of population size and genetic variation on the emergence of reproductive isolation. We used experimental evolution in replicated lines that differ in population size and standing genetic variability to examine whether sexual conflicts can lead to incipient reproductive isolation, and whether greater genetic variation or larger population size hastens reproductive isolation.

## 2. MATERIAL AND METHODS

### (a) Source population

Two replicate monogamous lines were established from an ancestral *Callosobruchus maculatus* population (Niamey, Niger) cultured on black-eyed beans (*Vigna unguiculata*) at 27°C, 32 per cent relative humidity and 16 L : 8 D photoperiod. Each generation we isolated virgin beetles immediately post-emergence. Virgins (less than 24 hours post eclosion) were paired and each pair was placed in a 40 mm Petri dish and observed until copulation had ceased. From these monogamous pairs, 60 singly mated females were placed together and transferred to approximately 400 beans for oviposition.

### (b) Experimental design

After 90 generations of enforced monogamy, polyandry was re-established in both lines by placing 60 newly emerging adults of each sex from each line on 400 beans. A third polyandrous line was created by combining 30 males and 30 females from each of the monogamous lines. In this crossed population, genetic variability should be greater, because 90 generations of isolation and drift would have promoted genetic differentiation and some loss of diversity from the two monogamous lines. All three polyandrous lines were allowed to expand exponentially for two generations, before we established 16 experimental populations. The crossed population (with larger genetic diversity) gave rise to eight lines at two different sizes (four small populations size = 50 individuals, four large population size = 5000 individuals; the density in terms of beans per individuals remained constant). We used the two other polyandrous lines to create another eight polyandrous lines with low genetic variability, four small (50 individuals) and four large (5000) (figure 1). This generated four treatments (small population size and low genetic variability; small population size and high genetic variability; large population size and low genetic variability; large population size and high genetic variability) each with four replicates. Males and females were housed together for their entire lifespan in all 16 lines.

To retain a constant population size and ratio of resources to beetles, we sieved and weighed the newly emerging adults in each generation and placed another 50 (for the small populations), or 5000 (for the large ones) individuals on new black-eyed beans (40 g for the small populations and 4000 g for the large populations).

### (c) Measurements

To reduce possible maternal and phenotypic effects, we standardized selection prior to the assay for all populations by housing beetles individually under standardized conditions—single mating and one egg per bean—for one generation. At generation 19, we performed a full crossing between the four populations within each treatment. We will refer to the crosses within populations (within treatments) as ‘sympatric’, and the crosses between populations (within treatments) as ‘allopatric’. Twenty pairs were used in each sympatric cross, and for allopatric crosses 10 females from each population mated to males from the alternative population. Virginity of beetles was ensured as above, and virgins (all less than 48 hours post eclosion) were paired according to the crossing design. Pairs were placed in

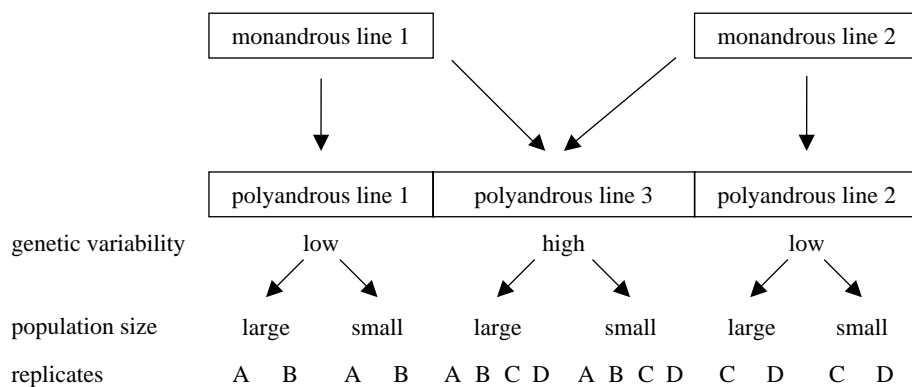


Figure 1. Experimental design showing the four treatments each with four replicates (A, B, C, D).

individual 40 mm Petri dishes and observed until copulation had started. We recorded the number of pairs that failed to copulate within 30 min as an indication of pre-mating isolation. After copulation had ceased, each female was transferred to a 90 mm Petri dish containing approximately 20 black-eyed beans and allowed to oviposit for 24 hours. We estimated the number of eggs laid during the first 24 hours by counting the eggs on one side of the beans using a Motic SMZ-168 microscope. For a subset of beans, we also counted the total number of eggs and found that both values were highly correlated ( $r=0.98$ ,  $t=16$ , d.f.=8,  $p<0.0001$ ). We subsequently counted the number of offspring emerging from eggs laid during the first 24 hours and measured female elytra length as a proxy for body size.

#### (d) Data analysis

We measured reproductive traits in all six possible allopatric crosses within a treatment (A×B, A×C, A×D, B×C, B×D and C×D; figure 1) and within all four sympatric crosses (A×A, B×B, C×C and D×D). Cross means were the unit of replication (rather than individual beetle pairs). This method still suffers from a degree of pseudoreplication (each line is used three times in the allopatric crosses), hence we analysed our data both by comparing measures of isolation (see below) based on all six allopatric measures and after designating two unique pairs within each treatment (i.e. A×B and C×D). Pairing the replicates is also useful, because for low genetic variability treatments, we crossed the two replicate lines that had originally been derived from the same monogamous line. Analyses were performed in R v. 2.7.1 (Ihaka & Gentleman 1996).

All the variables and all residuals were checked and found to be normally distributed (Kolmogorov–Smirnov test, all  $p>0.05$ ), with homogeneous variances (Levene tests, all  $p>0.05$ ). To test for the evolution of reproductive isolation, we compared the mean number of failed matings, the fecundity and the number of offspring between allopatric and sympatric crosses in each treatment using a general linear model (GLM) with elytra length as a covariate (for fecundity and the number of offspring only). To examine the effect of population size and initial genetic diversity on the evolution of reproductive isolation, we estimated an index of isolation per crossing and calculated the mean per female replicate ( $n$ =four replicates×four treatments) for fecundity 24 hours, offspring 24 hours and number of failed copulations as

$$I = \frac{\text{allopatric} - \text{sympatric}}{\text{sympatric}}$$

If any reproductive isolation has evolved, we expect  $I$  to be negative. We used a GLM with the mean isolation index across female replicates for the number of failed matings, fecundity or the number of offspring as dependent variables and population size, initial genetic diversity and their interaction as explanatory variables. Again, elytra length and longevity were used as covariates for fitness traits.

### 3. RESULTS

We found no influence of the type of cross (allopatric or sympatric) on the number of failed matings or on fecundity or number of offspring in any of our four treatments, using the analysis including all replicates or the paired analysis (all  $p>0.06$ ). However, reproductive isolation (as indicated by a negative

isolation index) tended to evolve faster in larger populations (figure 2), but this was statistically significant only for isolation measured through its effect on fecundity in the analysis with paired replicates (small populations (median ± s.e.):  $I=0.09 \pm 0.08$ ; large populations:  $I=-0.09 \pm 0.06$ ; table 1; figure 2). The initial genetic variability of populations had no significant effect on reproductive isolation.

### 4. DISCUSSION

None of our treatments had evolved significant reproductive isolation after 19 generations. However, the degree of isolation (low isolation index) was the greatest among larger populations, both for pre-mating isolation (number of failed matings) and post-mating isolation (fecundity and number of offspring produced in 24 hours). The larger number of mutations (if we assume 1/zygote/generation for fitness traits (Houle *et al.* 1992) we would expect approx. 48 000 in our larger populations) and/or the higher intensity of sexual selection in large populations thus seem to have driven faster evolution towards reproductive isolation. Our results are therefore consistent with Gavrillets' model (2000), and previous work on dung flies (Martin & Hosken 2003). In the short term, the response to selection is expected to be a function of the standing genetic variation segregating in a population. However, after a few tens of generations, the new variation generated by mutation is more likely to fuel evolutionary change and reproductive isolation (Mackay *et al.* 1994; Fuller *et al.* 2005).

The limited evidence for the evolution of reproductive isolation in our study could reflect the short duration of divergence, or reflect a constraint due to limited genetic variability in all our populations. The fact that small populations with low genetic variability tended to perform better in allopatric as opposed to sympatric crosses suggests these populations may have been suffering from inbreeding depression. But overall there was no significant effect of genetic variability in our models.

In such experimental evolution designs, isolation can be measured in a number of ways. We chose to cross the lines within treatments rather than use a common tester. This necessarily involves some level of pseudoreplication, which can be avoided only by pairing replicates only once (i.e. discarding some

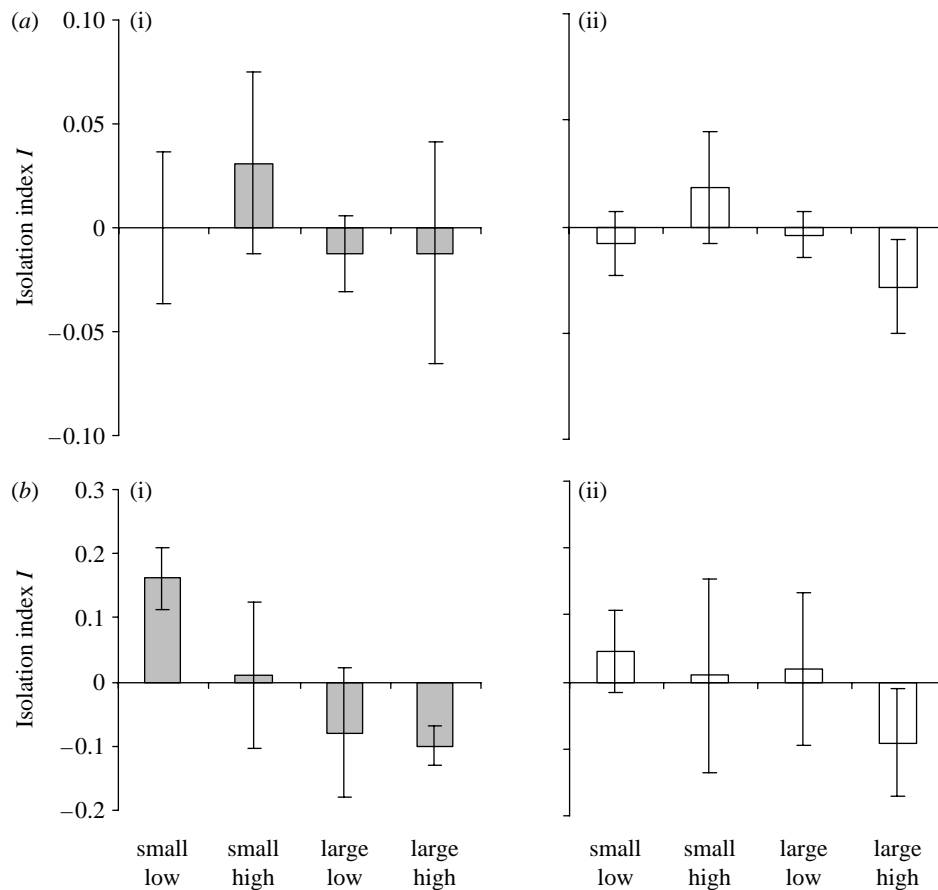


Figure 2. Isolation index  $I$  estimated on the medians ( $\pm$  s.e.) per female replicate for each treatment, for (a) mating failure and (b) fecundity; results presented for (i) paired (considering only eight crosses, grey bars) and (ii) unpaired (16 crosses, white bars) analyses. Small/large refers to initial population size and high/low to genetic variability.

Table 1. Effect of population size and initial genetic diversity on the evolution of reproductive isolation,  $I$  for the number of failed matings, fecundity and the number of offspring. Significant results are shown in italics.

	four replicates				paired replicates			
	sum of squares	d.f.	$F$	$p$ -value	sum of squares	d.f.	$F$	$p$ -value
<i>failed matings</i>								
population size	0.05	1	2.35	0.147	0.010	1	1.11	0.333
genetic variability	0.04	1	0.58	0.462	0.008	1	0.41	0.553
interaction	0.04	1	1.04	0.327	0.008	1	0.17	0.699
error	0.03	12			0.007	4		
<i>fecundity (eggs 24 hours)</i>								
population size	0.67	1	0.18	0.675	<i>0.11</i>	<i>1</i>	<i>7.43</i>	<i>0.034</i>
genetic variability	0.66	1	0.03	0.868	0.03	1	2.12	0.206
interaction	0.66	1	0.00	0.963	0.03	1	1.30	0.318
error	0.66	12			0.03	4		
<i>offspring 24 hours</i>								
population size	1.02	1	0.55	0.472	0.16	1	4.76	0.072
genetic variability	0.98	1	0.14	0.716	0.09	1	0.02	0.881
interaction	0.98	1	0.26	0.621	0.09	1	1.13	0.347
error	0.95	12			0.07	4		

information). Using a common tester addresses a different question, namely the divergence from this reference population. Moreover, the only really informative reference population would be the ancestral one, maintained in its ancestral state, which is often experimentally impossible. For these reasons, previous

studies of reproductive isolation driven by conflict coevolution have been based on crosses between replicates within treatments (Martin & Hosken 2003; Wigby & Chapman 2006; Bacigalupe *et al.* 2007; Dettman *et al.* 2008). Although each of our analytical approaches here have drawbacks, the results are

consistent and are significant in our most conservative analysis, suggesting a real effect of population size on the evolution of reproductive isolation as a by-product of sexually antagonistic coevolution.

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