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Sperm numbers vary between inter- and intra-population matings of the grasshopper *Chorthippus parallelus*

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Comparing the reproductive output of intra- and inter-population matings is the most common way to assess whether post-mating reproductive isolation is caused by genetic incompatibilities. Such genetic incompatibility can however, only assume that the quantity of the post-mating signals involved does not differ between intra- and inter-population matings. This assumption may not be true because sexual selection predicts reduced mating effort towards low-quality mates and in many circumstances, allopatric partners are low-quality mates. Post-mating efforts may, therefore, be reduced in inter- compared to intra-population matings. Here, I test this crucial assumption by studying variation in one post-mating trait, sperm number, in crosses of two parapatric grasshopper populations. In both populations, males transferred fewer sperm to allopatric than sympatric females. If such plasticity with respect to population is common in other post-mating traits, differences between inter- and intra-population crosses may be more frequently caused by differences in sperm number rather than gamete incompatibility. Additionally, I found that sperm numbers declined less rapidly in the female storage organ of allopatric than sympatric females but its rate differed markedly between populations. This is discussed with respect to female adaptations to male traits.

Keywords: *Chorthippus parallelus*; ejaculate size; genetic incompatibility; reproductive isolation; speciation; sperm number

1. INTRODUCTION

Fitness differences between inter- and intra-population matings are often ascribed to genetic incompatibilities between post-mating signal and receiver traits such as sperm–egg interactions or sperm–female interactions (e.g. Bella *et al.* 1992; Palumbi 1998; Parker & Partridge 1998; Rice 1998; Andrés & Arnqvist 2001; Nilsson *et al.* 2002; Swanson & Vaquier 2002). Less attention has been paid to the possibility that the post-mating traits may be plastic in relation to receiver origin (but note that Andrés & Arnqvist (2001) standardized one post-mating trait). However, such plasticity is predicted by sexual selection: if costly sperm production results in reduced ejaculate expenditure towards low-quality females, a fitness reduction in these females may be observed (Wedell *et al.* 2002). If inter-population crosses result in lower fitness than intra-population crosses, allopatric partners represent, by definition, low-quality partners. This may select for reduced investment into such partners. Two recent studies in a sexual/asexual fish species were consistent with that notion. Sperm production and sperm transfer were reduced in males reared in the presence of allopatric but not sympatric females (Aspbury & Gabor 2004; Schlupp & Plath 2005). If ejaculate sizes during copulation with an allopatric partner were commonly reduced in other species and resulted in female sperm limitation, fitness differences in inter- compared to intra-population matings may be more frequently caused than previously believed by numerical sperm differences rather than genetic incompatibilities.

I addressed this issue in a model system of reproductive isolation, the grasshopper *Chorthippus parallelus* (reviewed Butlin 1998; Tregenza *et al.* 2000*a*). Two parapatric subspecies *Chorthippus p. parallelus* (P) and *Chorthippus p. erythropus* (E) form a hybrid zone in the Pyrenees (reviewed Butlin 1998). Inter-population matings produce sterile male offspring (Virdee & Hewitt 1992). Therefore, allopatric partners represent low-quality individuals and males can be expected to discriminate against them. Allopatric partners were disfavoured in premating mate choice (e.g. Ritchie *et al.* 1989) and in fertilization (Bella *et al.* 1992). Here, I show that: (i) ejaculate sizes differ in sympatric and allopatric matings but (ii) sperm number manipulation by females results in little difference between such crosses.

2. MATERIAL AND METHODS

(a) *Rearing, mating protocol and sperm counts*

Nymphs collected from P and E populations sites (Puyvalador and Eyne (PU, EY; Butlin & Hewitt 1985)) were transported to a nearby laboratory. Newly eclosed adults were kept separate by sex and eclosion cohort and at a density of 10–20 individuals. Freshly cut grass blades of *Dactylis glomerata* were offered at 2–3 day intervals.

Individuals were randomly allocated to sympatric or allopatric matings and whether ejaculate size or sperm numbers after storage were to be investigated. Individual virgin females aged 4–6 days were daily offered a male in her cage. If copulation occurred, its duration was determined to the nearest 2 min interval. Individuals not mating within 3–4 h were returned to their respective cages. Any possible resulting age bias in the focal animals was circumvented by using male and female ages as covariates in the statistical model. The temperature during the mating protocols was between 29 and 30.5 °C.

I used an established sperm count protocol (Reinhardt *et al.* 1999; Reinhardt 2001) to measure ejaculate size. Potential manipulations of sperm by females did not affect the results because the entire spermathecal tract was removed from the female within 30 min of copulation. For further details see electronic supplementary material.

(b) *Sperm storage*

Females assigned to storage treatment were kept individually in cages provided with food and sand-filled cups for oviposition. The number of egg pods laid was included as a covariable in the model. On the assigned day, sperm were counted as described above. Storage was therefore entered as a binary variable (0/1; see electronic supplementary material for justification).

(c) *Body size*

A different sample of E and P males showed significant differences in femur length (a good measure of body size in *C. parallelus*—Tregenza *et al.* 2000*a*; E: 9.08±0.34 mm, $N=25$;

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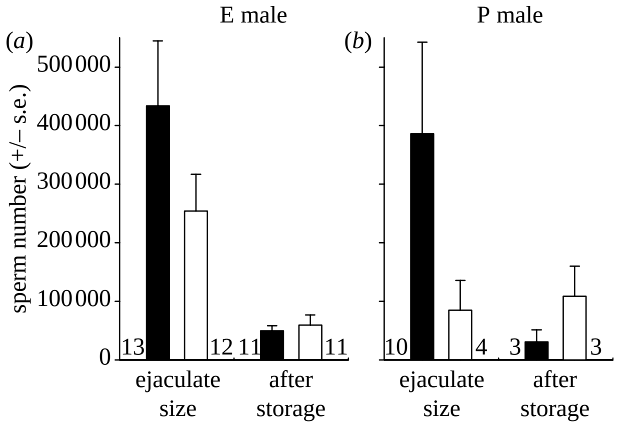

Figure 1. Effect of mating partner (sympatric—black bars, allopatric—white bars), storage duration in the female (*x*-axis) and male populations ((*a*) *C. p. erythropus* and (*b*) *C. p. parallelus*) on the numbers of sperm ($N=67$).

P: 9.96 ± 0.45 mm, $N=31$; $t=8.12$, d.f. $=54$, $p=<0.001$). In order to prevent masking population effects, I nested femur size within population in the present analysis.

(d) *Data analysis*

All data were analysed as generalized linear models using S-Plus. This enabled the incorporation of explanatory variables with very different distributions (Crawley 2002). The quasi-likelihood option, a log link function and an error variance increase with the square of the mean resulted in the best model (i.e. the model with the lowest residual variation—Crawley 2002). The goodness of fit of models are compared using log-likelihood ratio corrected for the number of degrees of freedom (known as the Akaike information criterion (AIC); e.g. Crawley 2002). Models with fewer parameters are selected if the model fit is not significantly lower than in more complex models (Crawley 2002).

3. RESULTS

Sperm numbers were explained by male age, size and population, mating type (intra- or inter-population) and sperm storage but not by female age or copulation duration (overall AIC 73.823). The model without these parameters (AIC: 70.623) explained 60.8% of the variation in sperm numbers (figure 1, table 1). Males of both populations inseminated fewer sperm when mated to females of the allopatric population (figure 1, table 1: mating type: $p<0.002$). However, sperm numbers declined less rapidly in allopatric than sympatric females (mating type × storage: $p<0.01$). This stronger decline in an allopatric than a sympatric environment was more pronounced for P than for E males (table 1: third order interaction: $p<0.001$, figure 1).

4. DISCUSSION

I found that: (i) fewer sperm were allocated to allopatric than to sympatric females and (ii) allopatric sperm numbers decreased at a lower rate from the sperm storage organ than sympatric sperm. Both findings have implications for theory and experimental design of reproductive isolation.

The fact that males mated at all with allopatric females suggests that males derive some benefits from such matings while allocating fewer sperm to allopatric females (Schlupp & Plath 2005, this study) suggests benefits of sperm economy. A potential benefit of sperm economy might be an increased sperm availability for future matings. This was not tested in the present paper but it was found that matings reduced sperm numbers in future matings for at least 1 day (i.e. approximately 5% of a male's lifetime): in three populations of *C. parallelus* (including E and P), ejaculate sizes declined from 100 to less than 10% with one recovery day between copulations but did not decline if there were 4 days in between (K. Reinhardt 1999 & 2002, unpublished data).

Table 1. Generalized linear model of the effect of male population (E or P), mating type (allopatric or sympatric), storage and several covariables on the number of sperm in the female spermatheca ($N=67$). (See text for model specifications.)

	slope ± s.e.	*t* value	*p*
intercept	1.265 ± 2.891	0.437	>0.50
male age	0.181 ± 0.065	-2.791	<0.01
number of egg pods	-0.702 ± 0.208	-3.382	<0.001
male population (E, P)	-8.343 ± 2.919	-2.858	<0.005
mating type	-1.169 ± 0.348	-3.357	<0.002
storage	-2.805 ± 0.882	-3.182	<0.005
E male size	0.285 ± 0.330	0.864	>0.50
P male size	2.169 ± 0.510	4.250	<0.001
male population × mating type	-0.757 ± 0.350	-2.162	<0.05
male population × storage	-2.480 ± 0.843	-2.942	<0.005
mating type × storage	1.481 ± 0.547	2.705	<0.01
male population × mating type × storage	2.037 ± 0.560	3.636	<0.001

While it is conceivable that males are largely in control of the number of ejaculated sperm, a study in guppies (Pilastro *et al.* 2004) demonstrated that females can influence ejaculate size as well. Female influences on ejaculate size in *C. parallelus* are largely unknown (Reinhardt 2001) and presently, I cannot exclude that, hypothetically, subtle morphological differences in the female genital tract reduce aspects of sperm transfer and, therefore, ejaculate size.

After copulation, hostile female responses towards sperm may represent female control over fertilization (e.g. Birkhead *et al.* 1993) which, in turn, selects for male traits to overcome such female adaptations (see e.g. Rice (1998) and Parker & Partridge (1998) for a reasoning). Part of the data presented here are consistent with such an interpretation. For example, in intra-population matings certain male or sperm traits that increase the storage of sperm may be selected for. If such traits impose costs on females, counteradaptations can be expected. However, if in inter-population matings non-coevolved sperm and female traits meet, a higher success of allopatric sperm may be observed (sexual conflict). Alternatively, non-adaptive population divergence may have been associated with divergence in female structures that recognize sperm (female divergence). Allopatric sperm would consequently be less well recognized and less attacked. While this is consistent with the slower decline of allopatric sperm in storage observed here, the same argument applies to a reduced recognition of sperm for fertilization. If fewer fertilizations lead to slower decrease in allopatric sperm numbers in storage, one would expect a lower number of allopatric sperm with a higher number of fertilization events. Observations in a central European population of *C. parallelus* did not support the latter notion because the decline in sperm number during storage was more strongly correlated to storage duration than to the number of fertilization events (Reinhardt *et al.* 1999). In the present study, the number of fertilization events, i.e. egg pods, was correlated with reduced sperm numbers, the lack of paternity analysis of the eggs did not allow me to separate between the sexual conflict or female divergence hypothesis.

C. parallelus is the classic example of assortative fertilization (Bella *et al.* 1992): E ejaculates competing against P ejaculates in the sperm storage organ of E females had a fertilization success of 53% but were outcompeted (24% fertilization success) by P ejaculates in P females (Bella *et al.* 1992). In addition to assortative fertilization, such a result may also arise if only E males ejaculated fewer sperm in allopatric matings. The data from the present non-competitive situation suggests otherwise: within a P female E sperm would have a 83.6% numerical representation against P sperm at ejaculation and 30.8% after storage (cf. figure 1). The resulting mean of 57.2% largely exceeds the 24% reported by Bella *et al.* (1992). The present data, therefore, suggest that the degree of assortative fertilization is even stronger than that expected from equal ejaculate sizes of E and P males.

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