Coevolution of non-fertile sperm and female receptivity in a butterfly

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Sexual conflict can promote rapid evolution of male and female reproductive traits. Males of many polyandrous butterflies transfer nutrients at mating that enhances female fecundity, but generates sexual conflict over female remating due to sperm competition. Butterflies produce both normal fertilizing sperm and large numbers of non-fertile sperm. In the green-veined white butterfly, Pieris napi, non-fertile sperm fill the females’ sperm storage organ, switching off receptivity and thereby reducing female remating. There is genetic variation in the number of non-fertile sperm stored, which directly relates to the female’s refractory period. There is also genetic variation in males’ sperm production. Here, we show that females’ refractory period and males’ sperm production are genetically correlated using quantitative genetic and selection experiments. Thus selection on male manipulation may increase the frequency of susceptible females to such manipulations as a correlated response and vice versa.

Keywords: sexual conflict; sperm competition; coevolution

2. MATERIAL AND METHODS

(a) Insect husbandry

Adult females were captured in Stockholm, Sweden. Thirty offspring from each female were reared in sub-groups of five on Alliaria petiolata leaves at 24°C on a 22L:2D cycle. On the morning after eclosion, individuals were weighed and given a colour mark to assign them to their family of origin. In total, offspring from 31 wild-caught females were reared. This procedure was repeated with 28 wild-caught females at a later date. The offspring were either assigned to half-sibling/full-sibling breeding design to calculate heritabilities (see below), or used to examine correlations between the sexes across full-sibling families (n = 25 families).

(b) Female refractory period

At 1 day of age, female offspring from the half-sibling (see below), or full-sibling families were haphazardly mated to a 1-day-old unrelated virgin male. Mating takes an average of 90 min. Following mating, females were provided with virgin (unrelated) males, A. petiolata for oviposition, and allowed to remate up to 10 days after their first mating. Females will rarely remate after this time (Wedell 2001). The refractory period (the number of days between first mating and second mating) was noted.

(c) Sperm counts

Male P. napi’s transfer two types of sperm in the spermatophore at mating: fertile, eupyrene, sperm and a large number of non-fertile, apyrene sperm. Non-fertile sperm are morphologically distinct from fertile sperm, and constitute more than 90 per cent of total sperm present in the males’ first spermatophore and may be responsible for the ejaculate consisting predominantly of non-fertile sperm. Here we examine the genetic architecture of female refractory period and non-fertile sperm transfer in P. napi to determine the potential for these traits to coevolve.

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(d) Family mean correlations

The relationship between the number of apyrene and eupyrene sperm present in the males’ first spermatophore and the female refractory period was examined across full-siblings from 25 families (mean of three sons and three daughters/family) using Spearman rank correlations corrected for ties.

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Table 1. Cross-sire family means from multivariate-nested ANOVA (r) and genetic correlations (in bold, mean ± s.e., upper panel). *p < 0.05; **p < 0.001; ***p < 0.0005; n.s. = not significant.

<table>
<thead>
<tr>
<th></th>
<th>male weight</th>
<th>refractory period</th>
<th>eupryene sperm</th>
<th>apyrene sperm</th>
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<tr>
<td>male weight</td>
<td></td>
<td>n.s.</td>
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<tr>
<td>refractory period</td>
<td>r = 0.125, p &gt; 0.3</td>
<td>0.501 ± 0.376*</td>
<td>0.480 ± 0.373*</td>
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<tr>
<td>eupryene sperm</td>
<td>r = 0.285, p &gt; 0.1</td>
<td>0.590*</td>
<td>0.922 ± 0.137*</td>
<td></td>
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<tr>
<td>apyrene sperm</td>
<td>r = 0.066, p &gt; 0.9</td>
<td>0.696**</td>
<td>0.848***</td>
<td></td>
</tr>
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</table>

3. RESULTS

(a) Female refractory period
Female refractory period was heritable in *P. napi* (h² = 0.772 ± 0.350, G = 4.244, p = 0.039; table 1). There was no effect of female weight (p > 0.2) or weight of the first male (p > 0.4, n = 101) on the refractory period.

(b) Male sperm production
The number of sperm transferred was heritable. This was true for both fertile (h² = 0.840 ± 0.411, G = 4.353, p = 0.003) and non-fertile sperm (h² = 0.424 ± 0.281, G = 2.074, p = 0.042). There was no relationship between the number of either fertile or non-fertile sperm and male size (table 1).

(c) Genetic correlations
The female refractory period is directly related to the number of non-fertile sperm stored (Cook & Wedell 1999; Wedell 2001). Full-sibling analysis revealed a positive relationship between the mean refractory period of females and the average number of non-fertile sperm (figure 1), but no significant relationship between the mean refractory period of females and the number of fertile sperm (r₁ = 0.30, z = 1.474, p > 0.1). This relationship was also confirmed by a genetic correlation between number of non-fertile sperm and the female refractory period in the half-sibling analysis (r₁G = 0.480 ± 0.373, p < 0.05). There was also a genetic correlation between the number of fertile sperm and the refractory period (r₁G = 0.501 ± 0.376, p < 0.05).

(d) Selection lines
The selection lines also provided evidence of a genetic correlation between male sperm transfer and female refractory period. Males from the line where females were selected for slow remating rates transferred significantly more fertile (F₁,5₁ = 19.437, p < 0.0001) and non-fertile sperm (figure 2). This indicates that selection on female refractory period can promote changes in males' sperm production, although genetic drift cannot be ruled out owing to lack of line replication.

4. DISCUSSION
Non-fertile sperm transfer and female refractory period is positively genetically correlated in *P. napi* as revealed by three separate studies: full-sibling family mean correlations, half-sibling quantitative genetic analyses, and the selection experiment reported here. Female refractory period is also genetically correlated with fertile sperm transfer, but was not correlated across full-sibling families. There is therefore scope for selection acting on females' receptivity and non-fertile sperm transfer, and evolutionary responses to selection in these traits. This genetic correlation is
probably owing to linkage disequilibrium caused by males producing many non-fertile sperm, increasing the refractory period of females that store many non-fertile sperm and vice versa. Sexual conflict over female remating rate in *P. napi* thus involves non-fertile sperm, as they switch off female receptivity (Cook & Wedell 1999), despite direct benefits from polyandry to females (Wedell et al. 2002), and may explain why non-fertile sperm make up 90 per cent of total sperm number.

Most analyses of sexual conflict over female mating explore situations when mating is costly to females. By contrast, *P. napi* females benefit from polyandry owing to male nutrient donations, although monogamous females live longer than genetically polyandrous females prevented from remating (Wedell et al. 2002). The mating conflict involves male manipulation (non-fertile sperm transfer) and female resistance (non-fertile sperm storage). It is unknown what the fitness costs are to males of producing many or few sperm.

Models exploring the potential for sexual conflict to generate antagonistic coevolution stress the importance of the shape of the females’ response to male manipulation. Exaggeration of male traits involved in overcoming female resistance is sensitive to the shape of the response in female resistance. If females increase the threshold amount of male stimulation (i.e. non-fertile sperm) required to switch off mating, this can generate cycles of coevolution. By contrast, if females evolve to become insensitive to males’ manipulation, they no longer exert selection on males and hence there is no evolution (Rowe et al. 2005). The outcome depends on the genetic variance in female resistance traits and the strength of natural selection acting on the trait(s) (Rowe et al. 2005). While it is clear that there is substantial genetic variation in the female refractory period in *P. napi*, it is not known to what extent storage of non-fertile sperm is subject to natural selection, but it is possible that non-fertile sperm may affect female overall fertility. The relationship between transfer and storage of non-fertile sperm is also complex. The numbers stored are substantially more variable than the number of non-fertile sperm inseminated (Wedell 2001).

The finding that female refractory period and sperm transfer are genetically correlated in *P. napi* is consistent with the previous findings showing that selection on female reproductive traits can directly affect male traits and vice versa (Martin & Hosken 2003). Coevolution between male and female reproductive traits (i.e. sperm production and storage) is unlikely to be affected by indirect genetic effects (i.e. females siring manipulative sons), as direct benefits are generally greater in magnitude (Cameron et al. 2003). The benefit to female *P. napi* of multiple mating in terms of increased fecundity vastly outweigh any potential benefit of siring manipulative sons that are better at reducing female receptivity. It is also unlikely owing to sperm production being a condition-dependent trait, as larval diet only affects males’ nutrient donation but not sperm numbers (Cook & Wedell 1996), and diet does not influence females’ likelihood of remating (Bergström & Wiklund 2002). Thus, sexual conflict is a likely candidate for the observed genetic correlation between female refractory period and male non-fertile sperm production in this butterfly.

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