Sexual comparisons in immune ability, survival and parasite intensity in two damselfly species

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Abstract

Recent evolutionary studies have suggested that females have a more robust immune system than males. Using two damselfly species (Hetaerina americana and Argia tezpi), we tested if females produced higher immune responses (as phenoloxidase and hydrolytic enzymes), had a higher survival (using a nylon implant inserted in the abdomen and measuring survival after 24 h) and fewer parasites (gregarines and water mites) than males. We also tested whether immune differences should emerge in different body areas (thorax vs. abdomen) within each sex with the prediction that only females will differ with the abdomen having a higher immune response than their thorax since the former area, for ecological and physiological reasons, may be a target zone for increased immune investment. Animals were adults of approximately the same age. In both species, females were more immunocompetent than males, but only in H. americana females were immune responses greater in the abdomen than in the thorax. However, there were no differences in survival and parasite intensity or the probability of being parasitised between the sexes in either of the two species. Thus, this study lends partial support to the principle that females are better at defending than males despite the null difference in parasitism and survival.

Keywords: Immune sexual dimorphism; Phenoloxidase; Hydrolytic enzymes; Survival; Parasitism; Damselflies

1. Introduction

In vertebrates, the fact that females mount better immune responses than males has been explained as a trading off effect of testosterone on males which increases the expression of courtship traits but suppresses immune ability (Folstad and Karter, 1992). This idea, however, has been strongly debated since the precise role of testosterone on immune responses is unclear (e.g. Roberts et al., 2004). Interestingly, the fact that females mount better immune responses than males is not restricted to vertebrates as this also occurs in invertebrates (reviewed by Rolff, 2002; Zuk and Stoehr, 2002; Schmid-Hempel, 2003). Given that invertebrates do not use testosterone and, nevertheless, show sexual differences in immunity, three hypotheses explaining this dimorphism have been put forward in which it is assumed that immunity is costly (e.g. Siva-Jothy and Thompson, 2002). The first hypothesis is based on the sexual selection pressure faced by males whose investment in immunity will be reduced as a consequence of investing more on secondary sex traits (Zuk, 1990; Zuk and Mcbean, 1996). The second hypothesis indicates that selection will operate on increasing female longevity as a way to maximise reproductive success via egg production (Rolff, 2002) giving, therefore, high priority to immunity (Rolff, 2002; Zuk and Stoehr, 2002). A more recent hypothesis suggests that rather than being fixed, immunity
is flexible attending to resource availability with sex specific changes in relation to reproductive opportunities (McKean and Nunney, 2005).

Some evidence in insects suggests that these cost-based hypotheses of the sexual immune dimorphism may explain male and female differences in immunity. For example, in *Drosophila melanogaster*, males that were housed with one female, mounted better immune responses when challenged with *Escherichia coli* than males housed with three females (McKean and Nunney, 2001). Also, males housed with males mounted better responses than males housed with females. These differences are presumably due to the negative effect of courtship behaviour and male–male competition for females on male immunity (McKean and Nunney, 2001).

Despite these sources of evidence, studies that have compared immune response differences and, furthermore, ability to resist an infection in both sexes have been criticised for a number of reasons. For example, it has been suggested that both components of the immune system (humoral and cellular) ought to be measured before conclusions are drawn, as males may have better immunity than females in some particular immune respects but not in others (Zuk and McKean, 1996; Adamo, 2004; Morales-Montor et al., 2004). Furthermore, differences in survivorship after an immune insult should be measured under similar conditions to control for differences in potential ecological pressures that both sexes experience (predators, food access, among others) (Zuk and McKean, 1996; Adamo, 2004). Another potential source of criticism is that selection for immunity may operate differentially in distinct regions of the body in both sexes in insects. For example, a better immune response in the abdomen compared to thorax is expected in females but not in males. Several explanations can be put forward for this: (a) the female abdomen is more likely to be damaged during mating than other body regions (e.g. Crudgington and Siva-Jothy, 2000; Blackenhorn et al., 2002; Reinhardt et al., 2003); (b) the abdomen is a first target zone for sexually transmitted pathogens (Knell and Weberley, 2004); (c) the abdomen is more frequently attacked by predators during oviposition, especially for terrestrial insects that lay eggs underwater (for example, odonates; Corbet, 1999); (d) the abdomen is the place where eggs are produced and, compared to sperm, eggs occupy a larger area so that immune responses should also be more largely distributed in this region; and (e) eggs should be equipped with a robust immune machinery against parasitoids and pathogens (e.g. Asgari et al., 1998). That there are regional differences in immunity in the body has been already documented in invertebrates and vertebrates (Zielinski and Pörtner, 2000; see also Adamo, 2004). However, to our knowledge, these differences have not been examined using an evolutionary perspective. Finally, another criticism is that age has been a confounding variable in many studies (i.e. variation in parasitic infection with age; e.g. Michalakis and Hochberg, 1994).

In this work we have tested the hypotheses that the female’s immune response and her survival after an immune challenge are higher than in the male. We used two species of damselflies, *Hetaerina americana* and *Argia tezpi*, to investigate these implications. We measured two immune parameters which provide a general picture of cellular and humoral response, phenoloxidase (PO) activity and hydrolytic enzymes. Although PO participates in non-immune functions (Cerenius and Söderhäll, 2004), the PO cascade also results in the melanisation and death of pathogens (Tzou et al., 2002; Cerenius and Söderhäll, 2004; Christensen et al., 2005), while hydrolytic enzymes are involved in the killing and degradation of micro-organisms (Cheng, 1992; Tzou et al., 2002; Hetru et al., 2003) or modifications of the molecular conformation surface of pathogens, and therefore favour recognition of phagocytic cells (Cheng, 1992; Cajaraville et al., 1995). Additionally, we also compared the types and quantity of proteases in both sexes as these are involved in the activation of PO cascade (Cerenius and Söderhäll, 2004).

First, we compared the immune response of both sexes. Our prediction is that females will have higher immune responses than males. Assuming potential sex-dependent differences in immunity in body regions in the same organism, we also looked for differences between the abdomen and thorax within each sex. Our expectation was that differences should only be observed in females with the abdomen having a higher immune response. We tested the survival of both sexes under a similar immune challenge in their natural conditions. The consequent prediction for this is that more females than males will survive. We also looked for differences in parasite intensity and occurrence in adults of both sexes controlling for host age. Although parasitism rate or probability of being parasitised are not the best indicators of differences in immunocompetence between the sexes due to different exposure to parasites (Rolff, 2002), we wanted to see whether these parameters showed similar sexual differences to those of immunity. We recorded the number of two endo- and ectoparasitic agents, respectively, protozoan gregarines and water mites. A number of studies have shown that these agents are the most common pathogens in odonate adults (reviewed by Corbet, 1999) causing a large negative impact on male and female fitness (for gregarines see for example Siva-Jothy and Plaistow, 1999 and Córdoba-Aguilar et al., 2003; for mites see for example Forbes, 1991; Forbes and Baker, 1991; Braune and Rolff, 2001, but see Andrés and Cordero, 1998). Our predictions were that males are more likely to be parasitised or that, when both sexes are parasitised, males have more parasites than females.

2. Materials and methods

2.1. Immune assessment

*H. americana* males and females were obtained from the Tehuixtla river (Morelos, Mexico) in May, 2004. *A. tezpi*
males and females were collected at La Buena Mujer Dam (Baja California Sur, Mexico) between May–June, 2004. The following sample sizes were collected: 20 males and 20 females of *H. americana* and 10 males and 10 females of *A. tezpi* for PO analysis; and 20 males and 20 females of *H. americana*, and 19 males and 19 females of *A. tezpi* for the analysis of enzyme activity and types. At the time of collection all animals were fully mature given that they showed reproductive activity (mating or oviposition activities). Given, also, that their body colour was not pale but shining is likely that we left aside young and old males and restricted our samples to an approximately similar age.

It is possible that immune differences between the sexes may be due to allometric relationships with body size. To check whether there were size differences between the sexes, we measured a sample of 15 males and 16 females of *H. americana* and 10 males and 10 females of *A. tezpi*. The length (to the nearest 0.01 cm) of the right anterior wing was measured as an indicator of body size.

2.1. Preparation of damselfly homogenate supernatant

Heads and wings were excluded. Each sample was homogenised with a polyethylene plastic micro pestle (during 5 min) in pre-cooled Eppendorf tubes containing 1 ml of Tris-buffered saline (TBS; 50 mM Tris, hydroxymethyl aminomethane, 1000 mM NaCl). The homogenate was centrifuged at 18,000 × g for 10 min at 4 °C in a Beckman centrifuge. The clear supernatant was removed and 1 ml of TBS was added to the pellet to be homogenised and centrifuged again for two times under the same conditions. Supernatants (3 ml) from each sample were pooled and used immediately in the assays or they were frozen (−80 °C) until use.

2.1.2. Assays for PO activity

PO activity was measured (in quadruplicate, obtaining an average of the four measurements) spectrophotometrically by recording the formation of dopachrome from L-dihydroxyphenylalanine (L-DOPA, Sigma). Fifty micro-litres of sample was mixed on a micro-well plate with 50 μl of L-DOPA (3 mg/ml of TBS) as substrate. Thereafter, 50 μl of buffer was added and optical density at 490 nm was recorded during 1 h every 5 min, using a micro-plate reader (Model 350, Bio-Rad). As sample blanks, 100 μl of buffer was mixed with either 50 μl of L-DOPA to detect possible spontaneous oxidation of this substrate by light, or 50 μl of a sample to eliminate sample colour. Enzyme activity was expressed as units, where one unit represents the change in absorbance min⁻¹ (Söderhäll and Hall, 1984). Haemocyte lysate supernatant from the shrimp *Litopenaeus vannamei* was used as the PO positive control. Since we found that the major PO activity took place at 5 min in both sexes of both species, we used the activity of this enzyme at this time. As a control we used a sample of each sex for both species. The absorbance of these samples was subtracted from the final absorbance to avoid the problem of high background. The change in absorbance was related to protein content and time.

We also compared the types and quantity of proteases (leucyl arylamidase, valyl arylamidase, cystyl arylamidase, trypsin and z-chymotrypsin) in both sexes (for their detection see below) for possible differences.

2.2. API ZYM system

An API ZYM commercial kit (BioMerieux, Inc.) was used for the detection of 19 hydrolytic enzymes (proteases: leucyl arylamidase, valyl arylamidase, cystyl arylamidase, trypsin, z-chymotrypsin; lipases: lipase esterase (C8) and lipase (C14); glycosidases: ß-galactosidase, ß-galactosidase, ß-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-ß-glucosaminidase, z-mannosidase and z-fucosidase; esterases: esterase (C1); and phosphatases: alkaline phosphatase, acid phosphatase and naphthol phosphohydrolase).

Sixty-five microlitres of each supernatant sample were added to the reaction strips and incubated at 37 °C for 4 h. Ten minutes after addition of reagents of the API ZYM kit at room temperature, the resulting colours were estimated and recorded from 0 to 5, according to a colour scale given by the manufacturer. Results were transformed to nM quantity of hydrolysed substrate. Enzyme activities were expressed as units, where one unit represents the hydrolysed substrate in nM/mg of protein. All measurements were performed in triplicate (three strips) using the average of the three.

2.3. Protein determination between sexes

The Bradford (1976) method was used to determine protein concentration in samples. Bovine serum albumin (Sigma®) was used as standard. To this, we added 20 μl of sample and 200 μl of buffer into individual wells of a 96-well plate. Forty microlitres of Bradford reagent was added and after 10 min, absorbance was read at 595 nm. As a control, we used 220 μl of buffer plus 40 μl of Bradford.

2.4. Survivorship after an immune challenge

The survival of both sexes after an immune challenge in *H. americana* (*N* = 30 for each sex) and *A. tezpi* (*N* = 19 males and 20 females) was recorded. The experiment was carried out in the natural habitats of these species. A previously disinfected (using 100% ethanol) nylon monofilament implant (3 mm length × 0.5 mm diameter) was totally inserted through the fourth abdominal pleura on the ventral mid-line using fine forceps. As a control, we used 30 males and 30 females of *H. americana* and 19 males and 20 females of *A. tezpi*. After the challenge, both experimental and control animals were left in an enclosure (2.5 × 2.5 × 2.5 m). The middle part of the enclosure was located in the river on one side while the other side occupied the river shore (on the vegetation). Given that this is the place where these animals usually live (the vegetation
for perching and feeding activities and the river for reproduction), is likely that they experienced the natural conditions they normally face (e.g. exposure to parasites). Damselflies were left inside the enclosure and survivorship was recorded after 24 h.

2.5. Parasite burden

Males and females of both species were collected monthly, *H. americana* from September 2004 to June 2005 and *A. tezpi* from November 2004 to February 2005, to determine whether there was seasonal variation in parasite intensity that may cause differences in parasite burden (e.g. Robb and Forbes, 2005). Soon after collection, animals were preserved in 70% ethanol. Damselflies were dissected by gently removing the entire gut and counting the gregarines attached to the gut lumen under a dissecting microscope. Mites attached to the cuticle or wing veins, or tracks of them, were counted. Previous studies have shown that mites detach from the damselfly host and that the scars left by the mite stylostoma can be seen by close inspection and can be easily counted (e.g. Forbes, 1991). Both the actual mites and scars can give a reliable picture of the parasite infection by these animals on the damselfly host (Forbes, 1991).

For the use of parametrical tests, parasite, enzyme, protease and protein concentration values were transformed using the formula $\sqrt{(x + 0.5)}$. Results are reported as mean $\pm$ S.D. unless stated otherwise.

3. Results

3.1. Body size differences

There were no size differences between the sexes in either species of damselfly (*H. americana* males: 2.61 ± 0.07, females: 2.59 ± 0.09; *A. tezpi* males: 2.42 ± 0.08, females: 2.46 ± 0.11; two way ANOVA $F_{3,61} = 2.1, P > 0.05$).

3.2. Phenoloxidase

There were differences in PO in overall abdomen and thorax between the sexes in both species (two way ANOVA $F_{3,56} = 55.21, P < 0.0001$; Fig. 1a and b) with females having more PO than males in *H. americana* (Tukey test $P < 0.05$; Fig. 1a) and *A. tezpi* (Tukey test $P < 0.05$; Fig. 1b). Also, there were differences in PO according to body areas (abdomen vs. thorax) between the sexes in both species (two way ANOVA $F_{4,115} = 105.6, P < 0.0001$; Fig. 1a and b); females had more PO in the abdomen (both species Tukey test $P < 0.05$; Fig. 1a). This was not the case for males (both species Tukey test $P > 0.05$; Fig. 1b).

As for proteases, both sexes in *H. americana* had the same types: leucyl arylamidase, valyl arylamidase, cystyl arylamidase, trypsin and $\alpha$-chymotrypsin. There were differences in both sexes in the amount of proteases in *H. americana* (ANOVA $F_{3,79} = 41.08, P = 0.0001$; Table 1). The post hoc comparisons indicated that females had more leucyl arylamidase than males (Tukey test $P < 0.05$) with rest of proteases being similar (Tukey test $P > 0.05$). In *A. tezpi*, only leucyl arylamidase, valyl arylamidase and trypsin and $\alpha$-chymotrypsin were found and they were present in both sexes. There were no differences between the sexes in each protease type in this species (ANOVA $F_{6,69} = 2.04, P > 0.05$; Table 1).

3.3. API ZYM system

There were differences in enzyme quantity in overall abdomen and thorax between the sexes in both species (two way ANOVA $F_{3,74} = 28.15, P < 0.0001$; Fig. 2a and b) with females having higher values than males in both species (Tukey test $P < 0.05$). There were also differences in abdomen vs. thorax enzyme values according to sex and species (two way ANOVA $F_{4,151} = 29.98, P < 0.0001$) in which the abdomen had higher enzyme values than thorax in females of both species (Tukey test $P < 0.05$; Fig. 2a and b). A similar difference was also present in *A. tezpi* males (Tukey test $P < 0.05$; Fig. 2a and b) but not in *H. americana* males (Tukey test $P > 0.05$; Fig. 2a and b).

3.4. Protein concentration

Protein concentration was not different between the sexes in both species (*H. americana* males: 4.36 ± 1.12, females: 5.11 ± 0.32; *A. tezpi* males: 0.87 ± 0.11, females: 0.82 ± 0.34; two way ANOVA $F_{3,55} = 1.19, P > 0.05$).

3.5. Survivorship after an immune challenge

Once the animals were left within the enclosure, they perched on walls and remained inactive. After 24 h, similar number of males and females of *H. americana* and *A. tezpi* survived with respect to control animals (Table 2).

3.6. Parasite occurrence and intensity

We collected 142 males and 37 females of *H. americana* and 75 males and 22 females of *A. tezpi*. There were no differences in the probability of being infected by gregarines according to sex (*H. americana* $X^2 = 0.8$ and *A. tezpi* $X^2 = 0.7$, both $P > 0.05$) and mites (*H. americana* $X^2 = 0.5$ and *A. tezpi* $X^2 = 1.3$, both $P > 0.05$). *A. tezpi* showed monthly variation in gregarines ($F_{3,96} = 11.65, P < 0.0001$) and mite rate ($F_{3,96} = 2.66, P = 0.05$) which was not the case for *H. americana* (gregarines, $F_{9,179} = 1.13, P = 0.34$; mites, $F_{9,179} = 0.62$, both $P > 0.05$). However, there was no sex-related difference in parasite intensity in both species (*H. americana* gregarines, $F_{1,179} = 0.037$, mites, $F_{1,179} = 0.15$, both $P > 0.05$; *A. tezpi* gregarines, $F_{1,96} = 0.04$, mites, $F_{1,96} = 0.87$, both $P > 0.05$).
4. Discussion

Both PO and hydrolytic (lysosomal) enzyme activities were higher in females than in males. These differences are unlikely to be explained by body size differences between the sexes. One plausible reason for the sexual differences may be that males and females were of different age, but this is unlikely given that we controlled for this factor in both sexes. We therefore hypothesise that the sexually dimorphic immune response in these two species are due to possible sexual differences in their life histories either via male investment on secondary sexual traits (Zuk, 1990; Zuk and McKean, 1996), female investment on increased longevity (Rolff, 2002) or plastic differences due to resource availability (McKean and Nunney, 2005).

Although there were no differences in protein concentration, there were differences in hydrolytic enzymes in both sexes in both species. Higher values of hydrolytic lysosomal enzymes in females than in males of both species seem to be explained by the role of enzymes in egg defence and yolk processing which is especially the case of proteases and lipases. For example, lysozyme-like activity has been found in the jelly coat of eggs of the echinoderm Paracentrotus lividus (Canicatti, 1990) and the eggs of the star fish Marthasterias glacialis (Stabili and Pagliara, 1994). Also, hydrolytic activity of 15 lysosomal enzymes was found in

![Fig. 1. Differences in PO in both sexes (abdomen + thorax) and between different regions within the same sex in Hetaerina americana (a) and Argia tezpi (b).](image)

**Table 1**

Pro tease values (in U/mg) in each sex in both species

| Proteases               | Hetaerina americana |  | Argia tezpi |  |
|-------------------------|---------------------|  |------------|------|
|                         | Males               | Females   | Males     | Females |
| Leucyl arylamidase      | 0.39 ± 0.53         | 7.84 ± 0.11 | 20.27 ± 26.65 | 92.17 ± 125.61 |
| Valyl arylamidase       | 0.43 ± 0.58         | 1.08 ± 0.94 | 20.26 ± 27.75 | 15.46 ± 23.72 |
| Cystyl arylamidase      | 0.4 ± 0.52          | 0.51 ± 0.56 | 7.71 ± 15.67 | 18.77 ± 31.29 |
| Trypsin and a-chymotrypsin | 0.36 ± 0.53     | 1.85 ± 1.83 |  |  |

Cystyl arylamidase was not found in *A. tezpi*. 

longevity (Rolff, 2002) or plastic differences due to resource availability (McKean and Nunney, 2005).

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eggs of the bivalve mollusc *Crassostrea gigas* (Luna-González et al., 2004). In addition, in *H. americana*, an immune challenge with bacteria increased the activity of the same 19 hydrolytic enzymes tested in this study in territorial and non-territorial males 24 h after the challenge (J. Contreras-Garduño, unpublished data). Although hydrolytic enzymes are involved in non-immune functions (e.g. Hetru et al., 2003), the above sources suggest that the hydrolytic enzymes we measured in our study may be important during immune function.

Recently, it has been stated that some tissues mount better immune responses than others (Zielinski and Pörtner, 2000; see also Adamo, 2004; Cerenius and Söderhäll, 2004). Our results in *H. americana* corroborate this, in that the abdomen seems better prepared than the thorax to resist an infection in females but not in males. Several explanations may account for these regional differences. The abdomen may be more prepared immunologically to resist sexually transmitted pathogens (Mantelli et al., 1998) which is especially the case for species whose females mate multiply and with different males as it is the case of the two species studied here (A. Córdoba-Aguilar, unpublished data). Another explanation comes from the oviposition mode in these animals in which females have to have their abdomen submerged to insert eggs into aquatic water substrates such as plants (Corbet, 1999). Attacks by predators (such as aquatic bugs, frogs or fish) during oviposition are common in odonates and they are usually directed to the abdomen (reviewed by Corbet, 1999). Another reason may be the extent of the area where gametes are produced. Although the production of sexual gametes in both sexes is in the same area (abdomen), eggs

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**Fig. 2.** Differences in hydrolytic enzymes in both sexes (abdomen + thorax) and between different regions within the same sex in *Hetaerina americana* (a) and *Argia tezpi* (b).

**Table 2**

Number of surviving individuals after 24 h in relation to the experimental treatment and damselfly species

<table>
<thead>
<tr>
<th>Species</th>
<th>Challenged animals</th>
<th>Control animals</th>
<th>$\chi^2$ ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td><em>Hetaerina americana</em></td>
<td>21</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td><em>Argia tezpi</em></td>
<td>15</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>
occupy a larger fraction of abdominal volume (sometimes more than half the number of abdominal segments: A. Córdoba-Aguilar, personal observation) compared to sperm (less than a fifth of the abdomen). Finally, selective pressure from parasitoids (e.g. Laplante, 1975) and pathogens in general (e.g. Siva-Jothy et al., 1995) on odonate eggs may also influence higher immune investment previous to oviposition. There was, nevertheless, no difference in immune function between the abdomen and thorax in A. tezpi. This result may be explained in terms of parasitic rates in both species by gregarines which are found in the abdomen only (Corbet, 1999). The intensity of gregarine infection is higher in both sexes of A. tezpi than in H. americana (males: r = 2.77, P = 0.0006; females: r = 2.93, P = 0.003). This may have favoured the development of higher immune responses in both males and females of A. tezpi. All these possibilities need to be investigated in detail. It is worth mentioning that our results, however, should be interpreted cautiously as PO is also involved in non-immune functions such as cuticular and egg tanning (Cerenius and Söderhäll, 2004) which may explain the sexual and regional immune and why both sexes did not differ in mortality in the survival experiment in the two species. Interestingly, the null difference in presence and quantity of three proteases in both species suggests that proteases activate the PO cascade equally in both sexes and that the main differences are expressed in the substrate or pathways after protease activity or that the three proteases tested here are not involved in PO activation. Although we found that H. americana females had more leucyl arylamidase than males, it is difficult to ascribe this as the reason why females produce more PO than males, as this was not observed in A. tezpi.

Our artificial infection protocol induced immune responses in at least two ways: by melanisation of the damage caused in the cuticle at the moment of insertion of the implant, and by “encapsulation” of the foreign agent (the implant inside the animal’s body). Since in both processes the activation of PO is necessary (Cerenius and Söderhäll, 2004), our experiment allowed for the immune sexual difference to act. This particular result means that females were better than males at repairing the damage by producing more PO than males. This sexual difference has been observed in other insects. One example occurs in the cricket Gryllus texensis, in which PO activity, haemocytne number, and susceptibility to the bacterium Serratia marcescens show differences between sexes and ages (Adamo et al., 2001). In the pre-reproductive period of these animals, immunocompetence in males was similar to that observed in females. When males reached the age at which they begin to display reproductive behaviour, PO activity and resistance to S. marcescens declined compared to females and younger males. In females, PO activity increased after the onset of sexual behaviour, relative to female nymphs and pre-reproductive adult females (Adamo et al., 2001). Although we had expected the results of the survival experiment to follow the sexual differences in immunity, there was no detectable difference in mortality. Differences in animal activity cannot account for this result as all individuals remained perched with little activity which included feeding behaviour. If investment in immunity is fixed, and if males invest less, then it would be expected that the trade-off would result in a decreased survival in this sex. McKean and Nunney (2005) have, however, suggested that immune response is plastic, limited by resource availability, rather than fixed which is contrary to previous hypotheses (Zuk, 1990; Zuk and McKean, 1996; Rolff, 2002). With abundant food, D. melanogaster males and females experienced no differences in immunity with females producing large numbers of eggs (McKean and Nunney, 2005). Interestingly, under these conditions, females experience reduced longevity (Fowler and Partridge, 1989; Chapman and Partridge, 1996). Immunity, however, became reduced when food provision was low and sexual activity was intense for females and males, respectively (McKean and Nunney, 2005). These adaptive changes in immunity follow physiological plasticity rules in which there will be sex-specific changes in immunity according to different ecological and socio-sexual situations. Studies in damselflies have indeed uncovered that both sexes experience apparently plastic changes in immunity in response to male territorial competition (Contreras-Garduño et al., 2006), oviposition behaviour (Siva-Jothy et al., 1998), predation (Joop and Rolff, 2004), parasitism (Joop and Rolff, 2004), food access (J. Contreras-Garduño, unpublished data), which partially explains observed ontogenetic changes in adult immunity (Rolff, 2001). This means that, possibly, mortality after an infection will not be necessarily different between the sexes as it will depend on the above factors which were unknown for our experimental animals. Manipulating food access in both sexes and again challenging them is the next step for testing the plasticity of immunity and its effects on survival in these animals.

We did not find sexual differences in parasitism in both damselflies which, although not necessarily predicted by the sexual immune dimorphism hypothesis (Rolff, 2002), illustrates potential ecological differences between the sexes. One explanation as for why females still have a similar parasite burden as males is that the parasites we quantified do not induce the immunity indicators we measured. This is unlikely as PO activity takes place after both gregarine (Siva-Jothy, 2000) and water mite attack (Joop and Rolff, 2004). Another explanation is that biases in infection can be caused by differential exposure to parasites in both sexes (e.g. Zuk and McKean, 1996; Schalk and Forbes, 1997). For example, it may be that females are more continuously exposed to parasites and, despite having a better prepared immune system, end up having similar parasitic rates to males. This hypothesis is likely to apply to gregarines as these are acquired via the small flies that serve as their hosts and which are preyed upon by damselflies
(Corbet, 1999). In this case, possibly females eat more gregarine-carrying flies than males given the fact that odonate females need to gain more mass than males during pre-maturity presumably because females need to devote more resources to egg production (Anholt et al., 1991). Parasitic biases in water mite infection can also be explained by differential habitat use as documented by Lajeunesse and collaborators (2004) in a study with two dragonfly species. Apparently, while perched on aquatic vegetation, adult males that wait for females become more exposed to emerging water mites than females which only visit these places for mating and oviposition (Lajeunesse et al., 2004). However, according to this explanation, males result more parasitised which is different to what we found.

Comparing parasitism between sexes does not seem the best way to investigate differential immune abilities in animals with innate immunity as a number of ecological causes have to be controlled for (Rolff, 2002). For example, Sheridan and collaborators (2000) did not find a trend in parasite biases in a meta analysis of arthropod studies in which the above causes were not considered. In fact, examining current data in gregarine and water mite parasitism in odonates do not reveal a gender bias pattern. Studies have indicated male (e.g. Lajeunesse et al., 2004), female (e.g. Åbro, 1996; Hecker et al., 2002; Forbes et al., 2004; Canales-Lazcano et al., 2005) or no biases at all (e.g. Rolff, 2000; Braune and Rolff, 2001; McKee et al., 2003, Robb and Forbes, 2005). Although age is important for understanding parasitic differences in odonates (e.g. Hecker et al., 2002) which has not been controlled properly in some of these studies, the fact is that there are gaps in the study of the ecological interaction between odonates and parasites which prevent the understanding of these differences.

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