The immunocompetence handicap hypothesis: testing the genetic predictions

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The immunocompetence handicap hypothesis suggests that the immune system competes for resources with sexually selected ornaments; variation in ornaments might reflect genetic variation for immunocompetence. We tested this genetic prediction by mating scorpionfly females to males differing in the expression of a condition-dependent ornament trait, saliva secretion, and then comparing offspring immunocompetence. We found several indications of an immunocompetence handicap in our study: females had superior immunocompetence compared with males, the different immune traits were positively correlated, and there were indications of genetic variation in immune traits. However, we found no significant difference in the immunocompetence of offspring derived from males differing in ornament expression, only a tendency for sons of ornamented males to possess slightly better immunocompetence. The estimated effect of fathers on offspring immunocompetence was rather small, but it might be a sufficient benefit of female choice, provided that the costs of choice are small. We conclude that the genetic benefit of female choice is small concerning offspring immunocompetence, but the immunocompetence handicap principle might nevertheless work in scorpionflies.

Keywords: sexual selection; good genes; immunocompetence; scorpionfly; *Panorpa*

1. INTRODUCTION

According to Darwin (1871), extravagant male ornamentation evolved in response to selection caused by female preferences for ornamented males as mates. However, Darwin was unable to explain why it should be adaptive for females to choose ornamented males. 'Good-genes' models of sexual selection (Trivers 1972; Zahavi 1975; Andersson 1994) propose that male ornaments indicate underlying quality, which can be inherited by the offspring, thereby improving their viability. This requires genetic variability in fitness-related 'quality' traits. It is still in dispute to what extent this is the case (Gustafsson 1986; Mousseau & Roff 1987; Pomiankowski & Møller 1995), because genetic theory predicts that strong selection tends to deplete genetic variability in these traits (Fisher 1930). However, females of many species act as if they are choosing males for 'good genes' (Johnstone 1995). Hamilton & Zuk (1982) proposed a way out of this dilemma: in spite of the close association with fitness, genes for disease resistance should always show considerable variation, because evolutionary changes in parasites and pathogens favour changes in resistance genes. Females choosing well-adapted males might thereby increase parasite resistance in their offspring. According to Hamilton & Zuk (1982), females can scrutinize males for their parasite resistance,

because males bearing a comparatively low parasite load can fully express sexually selected ornament traits such as bright plumage. There is some empirical evidence for a relationship between parasite load, male ornaments and female choice, but no such relationship was found in many other studies (Hamilton & Poulin 1997). The immunocompetence handicap hypothesis (ICHH) posits a possible reason for the absence of such a relationship (Folstad & Karter 1992; Wedekind & Folstad 1994): males carrying genes for superior immunocompetence (IC) might be able to allocate more resources from their immune system to costly ornament traits ('handicaps'), thus ending up with the same parasite load as more susceptible males. Females choosing ornamented males might therefore find mates with superior genes for IC, but average parasite load. This idea makes it essential to study female choice in relation to genetic differences in IC instead of phenotypic differences in parasite load. Offspring of ornamented males should have above-average IC. Until now, this main genetic prediction of the ICHH is untested.

Therefore, we investigated whether males of *Panorpa vulgaris* scorpionflies (Insecta: Mecoptera), with strong expression of a condition-dependent ornament trait, have offspring with superior IC. The examined male ornament trait is a nuptial gift in the form of salivary secretions produced during copulation. Female choice is based on these secretions, because females allow males producing many salivary masses to copulate longer. During copulation, sperm is transferred continuously and females use the sperm from different males proportionately for

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fertilization, so that males capable of producing many salivary masses have a higher reproductive success (Thornhill & Sauer 1992; Sauer *et al.* 1997, 1998). Males capable of producing many salivary masses sire offspring with enhanced competitive ability. Therefore, salivary secretion was interpreted as a genetic indicator trait or handicap according to Zahavi (1975) (Thornhill & Sauer 1992; Sauer *et al.* 1998). We now hypothesized that saliva secretion might be an immunocompetence handicap (ICH), because *P. vulgaris* females possess better IC than males (Kurtz *et al.* 2000). Such a dimorphism in the immune system is expected in the ICHH, because costly handicap expression in males should drain away resources from the immune system. In fact, the ICHH was initially stimulated by the frequent observation of a sexual dimorphism in IC in many vertebrate species, which might proximately be caused by the immunosuppressive effect of androgens (e.g. testosterone), hormones that stimulate the development of male characteristics used in sexual selection (Grossman 1989). Folstad & Karter (1992) suggested that androgens might be the proximate mechanism regulating the trade-off between ornament expression and IC. However, any other substance that exerts such an effect would fit the model assumptions, and therefore the model should also be applicable to invertebrates.

In experiments intended to show the genetic benefits of female choice, direct non-genetic as well as maternal genetic influences on the offspring are especially troublesome, because they might conceal genetic sire effects. We used an experimental design which eliminated these problems: females were each mated to two males differing in handicap expression. The IC of the offspring were analysed and genetic paternity analysis was used to assign the offspring to the correct father. This enabled pairwise comparison between half-sibs sharing the same mother and the same environment. To assess the IC of scorpionfly individuals, we used two traits, the phagocytic activity of haemocytes (Bayne 1990) and the haemolymph concentration of an antibacterial enzyme, lysozyme (Mohrig & Messner 1968). To reduce the phenotypic effect of differential resource allocation from the immune system to sexual ornamentation in adult males, larvae were used for the immune assays.

The 'good genes' which females might acquire by choosing ornamented males might be genes affecting IC directly or genes having more or less indirect effects on the immune system via body condition, e.g. genes affecting the rules by which condition is allocated to the immune system, the cost of ornament expression, or resource acquisition (Westneat & Birkhead 1998). Theoretically, the maintenance of genetic variability is most easily explained for genes affecting IC directly. However, empirical data needed for estimating the degree of genetic variability or heritability of IC are still scarce. We therefore analysed the IC of an additional scorpionfly generation and used parent-offspring regression to estimate the heritability of the two immune traits.

The main intention of this study was to investigate whether females genetically benefit from the choice of males producing many salivary masses by having offspring of superior IC. In addition, we examined further indications of an ICH: Do females possess superior IC compared

with males, and is there genetic variability in immune traits?

2. MATERIAL AND METHODS

(a) *Breeding of P. vulgaris*

P. vulgaris is the most common scorpionfly in western Germany, living in moist woods, shrubs and grassland communities (Sauer 1970). Dead arthropods are the main food of adults and larvae (Bockwinkel & Sauer 1994). In the laboratory, adults and larvae were provided with segments of last-instar mealworms (*Tenebrio molitor*) as food. Details concerning the breeding of *P. vulgaris* are given in Sauer (1977) and Thornhill & Sauer (1992). Briefly, the standard breeding protocol was as follows: adults collected in August 1997 in the field near Freiburg i. Br., Germany, were held pairwise in 10 cm × 10 cm × 7 cm plastic boxes containing peat-filled Petri dishes for egg laying. Larvae were reared on a 12 L:12 D photoperiod at 18 °C on moist paper tissues with *ad libitum* food at a maximum density of 15 individuals per Petri dish (12 cm in diameter). Larvae reaching the third larval instar were transferred to soil-filled, open-bottomed plastic cylinders (diameter 0.4 m) placed in the field, where they finished their development and overwintered. Adult flies emerging the next year were collected and used for the experiments.

In contrast to the standard breeding procedure, larvae assigned for the immune tests were reared on a photoperiod of 18 L:6 D and were not placed in the outdoor cylinders, but finished their larval development in the Petri dishes. When they ceased feeding in the last larval instar (L4), haemolymph was collected for the immune tests. Subsequently, the larvae were transferred to individual peat-filled plastic tubes (8 cm × 3.5 cm) and held at 18 °C in darkness for pupation. Adults, emerging after four to six weeks, were sexed and, if assigned for the heritability analysis, used for further breeding.

(b) *Estimation of handicap expression*

Handicap size of *P. vulgaris* males can be measured as the number of salivary masses produced during copulations. Saliva secretion is a condition-dependent trait, strongly influenced by an individual's ability to locate and defend food items (Bockwinkel & Sauer 1994). Prior to the estimation of handicap expression, we therefore kept the experimental males in semi-natural outdoor conditions with food limitation and competition, not protected from infectious organisms occurring in the field. Three populations of 60 males each, hatched from the field cylinders on the same day, were transferred to outdoor enclosures (150 cm × 70 cm × 70 cm) containing stinging nettle (*Urtica dioica*) twigs. The scorpionflies were supplied with *ad libitum* water, but food per enclosure and day was limited to 20 one-segment pieces of last-instar mealworms (*T. molitor*), randomly attached to the stinging nettle leaves, resulting in approximately 0.7 mg of food per male per day, which is comparable to field conditions.

After 12, 13 and 14 days of food competition, 59, 57 and 57 males survived in the first, second and third enclosures, respectively. Handicap expression was then estimated in two subsequent observations of mating behaviour. Mature, virgin females, bred under standard conditions, were used for the mating trials. To control for the influence of male choice (Sauer *et al.* 1998) only females of approximately equal body condition were used. Experimental males were individually transferred to 10 cm × 10 cm × 7 cm plastic boxes containing a layer of moist tissue. Females were randomly assigned to males, which usually

initiated copulation immediately. Typically, the copulation only continues for more than a few minutes if the male is able to provide a salivary mass to the female. After consumption of this nuptial gift, copulation only continues if the male is able to provide the female with further salivary masses; otherwise, the female disrupts the copulation. If males were not able to resume the copulation within ten minutes, the pair was separated. The male was then kept individually without food at 15 °C overnight and a second mating trial with another virgin female was performed on the subsequent day. We used the number of salivary masses produced during both copulations to estimate handicap expression and ranked males within each enclosure to divide each population into three approximately equally sized groups: males with high, medium and low handicap expression. Only 'high' and 'low' males were used for further experiments. They were fed *ad libitum*, and a haemolymph sample was taken for microsatellite analysis.

(c) *Haemolymph collection*

Haemolymph was collected, as described in Wiesner *et al.* (1998) and Gerken *et al.* (1998), from adults for microsatellite analysis and from larvae for microsatellite analysis and the immune assays. Up to 10 µl of haemolymph were collected from larvae and immediately drained into an autoclaved 1.5 ml reaction tube, which was pre-cooled on ice before use. One and a half microlitres were immediately diluted in 50 µl ice cold anti-coagulant buffer (see Wiesner *et al.* (1998) and Kurtz *et al.* (2000) for details). This sample was held on ice until it was used for the *in vitro* phagocytosis assay (the time on ice did not influence phagocytic activity). Three microlitres of the fresh haemolymph were used for the lysozyme assay. Afterwards, 500 µl sterile water were given to the remaining haemolymph. This sample, containing 1–5 µl of haemolymph, depending on the amount of haemolymph initially withdrawn, was used for DNA preparation for the microsatellite analysis.

(d) *DNA preparation and microsatellite analysis*

We extracted DNA from 1–5 µl of insect haemolymph using Chelex®100-suspension. Polymerase chain reaction amplification of the highly polymorphic microsatellite system *2Pv* was performed for paternity analysis. Details are given in Gerken *et al.* (1998) and Epplen *et al.* (1998).

(e) *Breeding of half-sib families for immunocompetence analysis*

In addition to the experimental males, microsatellite alleles were also analysed for virgin females. To establish half-sib families, the females were each paired to two males, both derived from the same enclosure, but one of which belonged to the 'high' handicap expression group, while the other one belonged to the 'low' group. Individuals were combined so that they differed in their microsatellite alleles, enabling unambiguous determination of paternity in the offspring. To achieve an approximately equal representation of both males in the offspring, the females were paired to both males for 90 min each. In a few cases, pairs were not willing to mate immediately and pairing was repeated on the subsequent days. Mated females were held individually for egg laying. After three days, a considerable proportion of females had not yet produced egg clutches. As this might have been caused by an insufficient number of copulations (*P. vulgaris* females are highly promiscuous and normally lay their eggs only after several copulations), the females were subsequently held together with their mates in

daily alternation. Eggs were collected and offspring reared as described above. When the larvae ceased feeding in the last larval instar, the IC of ten offspring of each female ($n = 48$) was tested. To control for environmental effects on IC, all ten individuals tested per family originated from the same egg clutch and were bred in the same Petri dish. In addition, the lysozyme and phagocytosis assays were performed on the same agar plate and culture vessel slide, respectively. If all ten offspring descended from the same father, a new set of ten offspring was analysed. Fifty-eight out of the 480 offspring (12.1%) failed to hatch to adults later on. One additional individual was excluded because determination of phagocytic activity was impossible due to coagulation of the cells in this preparation, leading to a sample of 240 female and 181 male larvae. Genetic paternity analysis was successfully performed for 464 out of the 480 offspring (96.7%). However, only half-sib families having same-sex offspring of both fathers could be used to compare the IC of offspring in relation to handicap expression of their fathers, resulting in 37 families with 189 female offspring and 30 families with 133 male offspring.

(f) *Lysozyme assay*

Lytic activity against *Micrococcus luteus* was determined in a 'lytic zone assay' modified from Mohrig & Messner (1968) and Wiesner (1992). Agar Plates containing 10 ml of a 1% agar L11 (Oxoid) with 5 mg ml⁻¹ freeze-dried *M. luteus* were prepared as described in Kurtz *et al.* (2000). Holes with diameters of 3 mm were punched in the agar layers and filled with 1.5 µl of 70% ethanol saturated with phenylthiourea (PTU) prior to use. After evaporation of the ethanol, traces of PTU remain in the wells and prevent melanization of the haemolymph. Three microlitres of haemolymph were put into each well. After incubation of the plates at 33 °C for 24 h, diameters of the clear lytic zones were measured with Vernier calipers. Standard curves were obtained by dilution series of lysozyme from hen egg white (Merck 5282, 100 000 units mg⁻¹). Based on the logarithmic connection to lysozyme concentration, diameters of lytic zones obtained from the haemolymph samples were converted to haemolymph lysozyme-like activities, i.e. ng µl⁻¹—equivalents of hen egg white lysozyme. The repeatability of this measure was estimated from preliminary experiments where two samples were obtained from each individual (ANOVA: coefficient of intraclass correlation $r_1 = 0.72$, $F_{24,25} = 6.168$, $p < 0.001$).

(g) *Preparation of haemocyte monolayers and in vitro phagocytosis*

The *in vitro* phagocytosis assay used here is based on assays previously described for vertebrate cells (Hed 1986) and insect haemocytes (Rohloff *et al.* 1994). As adhesion of particles to the haemocyte surface is not always followed by endocytosis, we used a 'quenching' procedure enabling the unambiguous distinction between attached and engulfed particles. The assay was newly adapted to *P. vulgaris* haemocytes and improved by an additional staining of cell nuclei. Details are given in Wiesner *et al.* (1998) and Kurtz *et al.* (2000). Each individual 1.5 µl haemolymph sample (prepared as described above) was used to prepare one monolayer. The phagocytic activity of these monolayers was quantified by offering 3×10^5 fluorescein isothiocyanate (FITC)-labelled hydrophilic silica beads (Merck LiChrospher® 100 NH₂, mean diameter 5 µm) for a period of 2 h at 33 °C (cf. Wiesner *et al.* 1998). Afterwards, the fluorescence of non-ingested particles was 'quenched' with trypan blue, a dye which does not enter living cells, and the haemocyte nuclei were

fluorescently labelled with the DNA-specific fluorochrome 4',6-diamidino-2-phenylindole (DAPI). Monolayers were then fixed with 4% formaldehyde, embedded in Kaiser's glycerol gelatine, mounted with cover-slips, and stored in a refrigerator until microscopic evaluation.

To determine the haemocyte count and phagocytic activity, haemocytes and ingested silica beads were counted using a digital imaging system. A Photometrics Sensys CCD KAF 1400 camera attached to a Leica DM epifluorescence microscope was used with the Signal Analytic IP Lab Spectrum 3.11 software on a Power Macintosh 8100/80 workstation. Four counting areas of 0.63 mm^2 each (resulting in 8% of the whole culture area), but at least 1000 cells per monolayer were analysed, alternating between the fluorescence activation of FITC and DAPI. Background subtraction using the 'image ratio' procedure of the IP Lab Spectrum 3.11 software was used to enhance the contrast under DAPI irradiation. Digital image analysis enabled the efficient determination of the amount of haemocytes and phagocytically active haemocytes, but it was not possible to separate particles lying close together within one cell. Thus, the number of ingested particles per phagocytically active haemocyte could not be determined. However, previous experiments, where manual counting was performed, had shown that the number of ingested particles per phagocytically active haemocyte was of minor importance for the total phagocytic capacity (Kurtz *et al.* 2000). Thus, it seemed reasonable to dispense with this variable in favour of the considerably faster and more convenient digital image analysis. The number of phagocytically active haemocytes per microlitre of haemolymph was used as an estimate of an individual's phagocytic activity (repeatability: $r_1 = 0.66$, $F_{6,7} = 4.920$, $p = 0.028$).

(h) Heritability analysis

To estimate the heritability of the immune traits, resemblance of parents and offspring was analysed. Fifty pairs were taken from the individuals already tested for their IC (see §4(e–g)). Concerning their IC, pairs were randomly chosen, but no related individuals were used, i.e. paired males and females always descended from different half-sib families. Offspring were reared and the IC of six offspring per family was analysed as described above.

(i) Statistics

Statistics were performed according to Sokal & Rohlf (1995), using the SPSS 8.0 and JMP 3 software. Quoted significance values are for two-tailed tests. The level for significance was set to $p < 0.05$.

Lysozyme-like activity and phagocytic activity were normalized (Kolmogorov–Smirnov test with Lilliefors correction, $p > 0.2$) using logarithmic and square-root transformations, respectively. Within the half-sib families, means for male and female full-sibs were calculated. Differences in lysozyme and phagocytosis between offspring of the two types of males were then tested with mixed-model two-way analyses of variance (ANOVA), including the fathers' handicap expression (high–low) as a fixed factor and family as a random factor. To test for a difference in both immune traits simultaneously, a MANOVA model was calculated using the 'identity' design of JMP 3. The raw effect sizes with their 95% confidence limits are given as percentage change resulting from mating with highly ornamented males compared with random mating. For this calculation, parameter means were transformed back to the untransformed scale. Power details (Cohen 1988) were obtained with the JMP 3 software. The

Figure 1. Effect of male handicap expression on offspring immunocompetence ((a) lysozyme-like activity, given as nanograms of lysozyme-equivalents per microlitre of haemolymph, $\log_{10}$ transformed; (b) phagocytic activity, given as phagocytically active haemocytes per microlitre of haemolymph, square-root transformed). Each bar represents the same-sex offspring of one half-sib family. The bars show the difference between the offspring of fathers with high and low handicap expression, with positive values indicating the superior immunocompetence of offspring of highly handicapped males. See table 1 for a detailed statistical analysis.

least significant sample and effect sizes necessary to reach a power of 0.80 were calculated for an α -level of 0.05, using the standard error obtained from this study.

For the estimation of heritability, a parent–offspring regression was performed for the sexes separately. To control for unequal means and variances between the sexes and generations, values were standardized by z -transformation before regression, as suggested by Lynch & Walsh (1998) for differences between the sexes. The heritability was then estimated as twice the linear regression coefficient (Falconer & Mackay 1996).

3. RESULTS

Both immune traits tested were sexually dimorphic, with females possessing a significantly more powerful immune system (two-way mixed-model ANOVA including family as a random factor: $F_{1,372} = 7.162$, $p = 0.008$ for haemolymph lysozyme-like activity; $F_{1,372} = 7.627$, $p = 0.006$ for phagocytic activity; MANOVA: Wilks' $\lambda = 0.972$, $F_{2,371} = 5.403$, $p = 0.005$ for both traits). Further analysis was therefore performed for the sexes separately. The two immune traits were positively correlated in males (Pearson $r = 0.264$, $p < 0.001$, $n = 181$) and females (Pearson $r = 0.280$, $p < 0.001$, $n = 240$).

The main genetic prediction of the ICHH states that offspring of males with highly developed handicap traits have superior IC compared with males with low handicap expression. We therefore compared the two immune traits between offspring of the two types of males within half-sib families (figure 1). No significant differences were found (table 1). There was a tendency for the sons of males with highly expressed handicaps to have higher lysozyme activity. A similar trend was observed for phagocytic activity, but was far from significance. The tendencies were even weaker and pointed to the opposite direction for daughters.

Table 1. *Effect of male handicap expression on offspring immunocompetence*

(Results of mixed-model two-way ANOVAs with offspring haemolymph lysozyme-like activity ($\text{ng } \mu\text{l}^{-1}$; $\log_{10}$ transformed) or phagocytic activity (active cells μl^{-1} ; square-root transformed) as the dependent variables and fathers' handicap expression as the source of variation. Family means of same-sex full-sibs were used for the analysis. As each female was paired to both types of males, family was included as a random variable, which includes the effect of mother and common environment shared by the offspring, and was significant ($p < 0.01$) in all cases. Effect sizes are given as percentage change in offspring immune traits (transformed back) resulting from mating with highly ornamented males, compared with random mating. There was no significant effect of male ornament on both immune traits: MANOVA, sons: Wilks' $\lambda = 0.89$, $F_{2,28} = 1.67$, $p = 0.206$; daughters: Wilks' $\lambda = 0.94$, $F_{2,35} = 1.05$, $p = 0.361$.)

		means $\pm$ s.e.						effect size (%)			power analysis		
dependent variable	offspring sex	handicap of fathers		analysis of variance				confidence limit			power = 0.8 for		
		low	high	SS	d.f.	<i>F</i>	<i>p</i>	mean	lower	upper	power	<i>n</i>	effect (%)
lysozyme	sons	1.13 $\pm$ 0.04	1.21 $\pm$ 0.04	0.086	1, 29	3.13	0.088	9.1	-1.4	20.7	0.40	77	15.6
	daughters	1.26 $\pm$ 0.04	1.21 $\pm$ 0.04	0.051	1, 36	1.85	0.182	-5.8	-14.0	3.0	0.26	158	-13.8
phagocytosis	sons	56.0 $\pm$ 3.0	59.1 $\pm$ 3.1	146.5	1, 29	1.62	0.213	5.5	-3.3	14.7	0.23	147	12.8
	daughters	66.1 $\pm$ 2.7	63.9 $\pm$ 3.1	88.4	1, 36	0.63	0.431	-3.3	-11.6	5.3	0.12	460	-12.6

With a parent-offspring regression, we estimated the heritability of the immune traits for males and females separately (figure 2). In haemolymph lysozyme activity, a significant heritability of $h^2 = 0.75 \pm 0.28$ ($F_{1,45} = 7.413$, $p = 0.009$) was found for mothers on daughters. The heritability estimate for mothers on sons was about half the size, but not significantly different from zero. No significant heritability of the father's lysozyme activity was observed in either sons or daughters. For phagocytic activity, no genetic effect of mothers on offspring was observed, but fathers had a highly significant influence on the phagocytic activity of their daughters ($h^2 = 0.83 \pm 0.27$, $F_{1,45} = 9.211$, $p = 0.004$) and sons ($h^2 = 0.77 \pm 0.27$, $F_{1,46} = 8.069$, $p = 0.007$).

4. DISCUSSION

Our study gave some indications that the ICHH might work in *P. vulgaris* scorpionflies: females possess a more powerful immune system than males, the different measures of IC are positively correlated, and there seems to be genetic variation in the immune traits studied. Such genetic variation in immune traits is a necessary condition for the ICHH. The positive correlation between the two immune traits is in agreement with the ICHH, which posits a general, condition-dependent trade-off between the immune system and other traits. Otherwise, we would expect a negative correlation between different immune parameters, because of a trade-off within the immune system (Møller *et al.* 1999; Kurtz *et al.* 2000). The superior IC of females hints at similarities to vertebrate immune systems, where the frequent observation of a sexual dimorphism in IC initially led to the proposal of the ICHH.

Despite these indications, our results failed to support the main genetic prediction of the ICHH: offspring of scorpionfly males with high expression of a condition-dependent ornament trait, saliva secretion, were not significantly superior in the two immune traits. At least, there was a tendency in the expected direction, but this applied to sons only. Our study is the first, to our knowledge, testing offspring IC in relation to the fathers'

handicap expression. The few other studies in this field focused on the effect of mate choice on offspring parasite load (Møller 1990) or major histocompatibility complex genotype (Von Schantz *et al.* 1996), and gave support to the Hamilton-Zuk hypothesis (Hamilton & Zuk 1982). Why was no significant relationship found between ornament expression of fathers and IC of offspring in our study?

First, the absence of a statistically significant effect does not necessarily mean that there is no effect, because the statistical power of our study, i.e. the probability of getting a statistically significant result given that there is an effect (Cohen 1988), might have been insufficient. In our study, power ranged from 0.12 to 0.40, depending on the immune trait and sex under consideration (table 1). To reach a power of 0.80, sample sizes between 77 and 460 half-sib families would have been necessary. Assuming a power of 0.80 and the sample sizes used in this study, we can estimate which change in offspring immune traits we would have been able to detect. Comparing choosy females that mate with highly ornamented males with females that mate at random, we would have been able to detect a 15.6% increase for the strongest observed effect, lysozyme activity of sons. The estimated mean effect was a 9.1% increase for this trait, but the maximum was 20.7%, as estimated from the 95% confidence limit (table 1). From the latter value, it can be concluded that the genetic benefits of female choice, regarding the offspring immune traits studied here, are unlikely to be much greater than 20%. It should be noted, however, that even smaller genetic benefits might be relevant for the evolution of female choice, provided that the costs of mate choice are sufficiently small (Kirkpatrick & Barton 1997; Alatalo *et al.* 1998; Møller & Alatalo 1999). In *P. vulgaris*, where mate choice is cryptic by means of copulation duration, females copulate with many males during their lifetime and copulations are much longer than necessary for sufficient sperm supply (Sauer *et al.* 1998). Therefore, costs of mate choice primarily result from the potentially higher risk of predation during copulation, where pairs are easily visible and comparatively sluggish. These costs

Figure 2. Heritability of immune traits as estimated from parent-offspring regression. Immune parameters are as in figure 1, but additionally z -transformed. Heritability estimates, based on unweighted means of one to six offspring per family, are given with their standard errors (** and linear regression lines indicate heritability estimates significantly different from zero, $p < 0.01$). (a) Lysozyme, (b) phagocytosis.

might be partly offset by the resources gained from nuptial gift consumption, especially when females are undernourished, but the benefit of nuptial gift consumption is only weak in *P. vulgaris* (H. Kullmann and K. P. Sauer, unpublished data). There seem to be no further costs of mate choice for *P. vulgaris*, like extensive mate searching behaviour or pheromone production (K. P. Sauer, unpublished data). Thus it is likely that female choice is not very costly in *P. vulgaris*, so that even small genetic benefits would be sufficient to compensate for the costs of choice.

Apart from the possibly insufficient statistical power of our experiment, a second reason for the absence of a significant relationship between ornament expression of fathers and offspring IC could be that the types of 'good genes'

existing in scorpionflies might not have been detectable through our experiments. Westneat & Birkhead (1998) pointed out that the 'good genes' which females might acquire need not necessarily be genes affecting immunity directly. This is especially important for our study, because we only studied immune traits, but no other life-history traits or ornament expression in the offspring. Therefore, we would have been unable to detect any benefits of female choice in cases where the link between immunity and 'good genes' is indirect. For example, if there is genetic variation for the rules by which condition is allocated to ornaments versus IC, then females choosing highly ornamented males will get highly ornamented offspring with lower IC. In this case, females would only benefit from their choice if the impact of pathogens decreases in the following generation. Changes in the optimal balance between IC and ornaments due to shifts in the presence of pathogens or in their effects on hosts might contribute to the maintenance of genetic variation for the rules by which condition is allocated to ornaments versus IC (Westneat & Birkhead 1998). The link between mate choice for 'good genes' and IC might be even more indirect, for example, if there is genetic variation in traits affecting the efficiency of resource acquisition or ornament expression, leading to variation in condition and therefore in IC (Westneat & Birkhead 1998). However, the maintenance of genetic variation would be much more difficult to explain in the face of strong selection on such traits. More likely, genetic variability in immune traits could lead to differences between individuals in foraging ability (e.g. due to the effects of illness) and in ability to invest acquired resources into ornaments instead of immune defence. This was the reason for focusing on female choice for 'good genes' directly influencing immune traits in this study. However, it should be noted that environmental influences on condition and therefore on ornament expression are most likely very strong compared with underlying genetic variation. The mainly accidental nature of finding food items might have a much stronger influence on condition than the genetically based ability to invest the acquired resources into ornaments versus immune traits. A further difficulty in testing the genetic prediction of the ICHH should be mentioned: concerning immune traits, 'good genes' might not necessarily be those leading to a maximum immune response, but those leading to an optimal regulation (Siva-Jothy & Skarstein 1998; Owens & Wilson 1999; Von Schantz *et al.* 1999). For example, genes leading to high phagocytic activity might be 'good' in times of high parasite pressure, but otherwise infer high costs due to immunopathological effects.

Significant correlations between parent and offspring IC were observed in this study, but heritability estimates differed largely between the sexes. In one trait, lysozyme activity, maternal effects might play a role or genes regulating haemolymph lysozyme activity might be sex-chromosomal. While there was no effect of maternal phagocytic activity on offspring, a significant effect was observed for fathers, leading to high heritability estimates. Direct non-genetic effects transferred from fathers to the offspring via salivary secretions or sperm (which are very large in *P. vulgaris*) could lead to such an effect. More likely, the heritability of phagocytosis is obscured in females by additional sources of phenotypic variation. For

example, if the maximum phagocytic capacity is the underlying heritable trait, and females were not working to capacity in the parental generation, then heritability might be visible in males only. However, these explanations are speculative and deserve further investigation. Interestingly, Möller (1990) found in a cross-fostering study that, after controlling for the possible effect of assortative mating, the mite load of offspring of barn swallows was positively correlated to that of fathers, but not of mothers. Maybe, sex differences in the heritability of parasite resistance and IC are more widespread than currently thought. There are astonishingly few studies on the heritability of general IC. Fevolden *et al.* (1994) reported medium magnitude heritability of lysozyme activity in the Atlantic salmon ($h^2=0.19$); Kean & Cahaner (1994) demonstrated that multi-trait IC substantially responded to selection within seven generations in chickens and reported a heritability estimate of $h^2=0.05$ for phagocytic ability. More often, genetic variability in traits associated with resistance to specific parasites is tested and the given heritability estimates are often quite high, typically ranging from 0.10 to 0.50 (Goater & Holmes 1997). There are few studies on invertebrate immune reactions, which usually demonstrate similarly high heritabilities (Carton & Nappi 1991; Fellowes *et al.* 1998; Kraaijeveld & Godfray 1999). This gives support to the assumption that host–parasite coevolution is a source of genetic variation in immune traits. The uncovering of genetic variation in immune traits helps to identify the ‘good genes’ which choosy females might acquire.

In conclusion, our study supports several aspects of the ICHH, but ‘good genes’ effects on offspring IC were at best small. However, these small genetic benefits might be sufficient for female choice. The predictions of the ICHH depend on the type of ‘good genes’ involved. Further studies on the genetic predictions of the ICHH, taking these aspects into consideration, can lead to new insights into the evolution of female mate choice.

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