Genetic variation in infestation with a directly transmitted ectoparasite

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Abstract

Genetic variation in levels of parasitism of hosts is an underlying assumption of studies of coevolution, but few such estimates are available from the field. We studied genetic variation in the abundance of the chewing louse *Hirundoecus malleus* on its barn swallow host *Hirundo rustica*. These parasites are directly transmitted and a test of genetic variation of parasite abundance would thus provide a particularly strong test. The prevalence and the abundance of the chewing lice did not differ significantly between adult male and female hosts. The resemblance in parasite intensity of *H. malleus* of offspring and their parents was positive and highly significant, and an analysis of extra-pair paternity in the host allowed partitioning of this resemblance between genetic and common environment effects. There was no significant resemblance in parasite intensity between extra-pair offspring and their foster parents, although the resemblance remained for within-pair offspring. This provides evidence for the abundance of directly transmitted parasites having an additive genetic component. We found no evidence of common environment effects as parents did not resemble each other with respect to lice abundance.

Introduction

Parasites have been hypothesized to play an important role in areas as diverse as the evolution of sex, host life history, sexual selection, dispersal and many other phenomena (e.g. Hamilton, 1980, 1986, 1993; Hamilton & Zuk, 1982; Hamilton *et al.*, 1990; Frank, 1991; Folstad & Karter, 1992; Hochberg *et al.*, 1992; Olivieri *et al.*, 1995). Central to this interest in parasites is their ability to coevolve with their hosts, thereby generating continuing reciprocal evolutionary interactions between the two parties (Futuyma & Slatkin, 1983; Thompson, 1994). Coevolutionary scenarios are based on the assumption that the negative impact of the parasite on host fitness (parasite virulence) selects for increased resistance in the host, which in turn may select for increased virulence in the parasite (e.g. Futuyma & Slatkin, 1983; Thompson, 1994). Thus, such scenarios are based on assumptions

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concerning the ability of hosts and parasites to show a micro-evolutionary response to selection because of the presence of additive genetic variance in resistance and virulence.

Although coevolution has gained increasing interest from the scientific community in recent decades, there is relatively little information on the importance of environmental and genetic variance as determinants of parasite virulence and host resistance, particularly when it comes to field studies of free-living organisms. For example, a recent review of the genetics of host resistance in animals only reported two studies investigating the resemblance in parasite abundance of offspring and their parents (Sorci *et al.*, 1997). A few more estimates exist for insects (Polak, 2003). Such estimates consistently suffer from problems of partitioning of variance components among the additive genetic variance, common environment effects and maternal effects (e.g. Falconer and Mackay, 1996; Roff, 1997; Mousseau & Fox, 1998). This is even the case when offspring of single broods are split and reared in two different environments (Møller, 1990), because females may affect the quality of eggs before cross fostering has been made (Blount *et al.*,

2000). Vertebrate mothers consistently allocate immune substances such as immunoglobulins and antibodies and biochemicals such as carotenoids to eggs or offspring (e.g. Brambell, 1970; Kowalczyk, 1985; Blount et al., 2000; Saino et al., 2001, 2002a–c). This suggests that offspring performance in terms of parasite resistance may be strongly affected by maternal effects. The two studies on mite abundance of barn swallow Hirundo rustica hosts (Møller, 1990) and tick abundance of kittiwake Rissa tridactyla hosts (Boulinier et al., 1997) both attempted to control for potential maternal effects. We assessed whether aspects of maternal phenotype and the common breeding environment could account for similarity in parasite abundance. There was no evidence of such maternal effects in any of these studies.

The aims of the present study were to investigate genetic variation in the abundance of a common ectoparasite, Hirundoecus malleus, which is directly transmitted among its barn swallow H. rustica hosts. Similarity among relatives in parasite abundance was used as an estimate of additive genetic variance in underlying susceptibility to this chewing louse. As these ectoparasites are directly transmitted, any similarity among relatives in parasite abundance may arise because of a common environment. We partitioned the effect of additive genetic variance from common environment and maternal effects by exploiting the fact that approximately a third of all offspring of the host are extra-pair offspring. Hence, we predicted a resemblance in parasite abundance for adult males and their true offspring, but not for extra-pair offspring if the main determinant was genetic, and a similar degree of similarity for the two categories of offspring if the main determinant of parasite abundance was the common environment.

Material and methods

Study organisms

The chewing louse H. malleus is a Phthiraptera belonging to the sub-order Amblycera. It lives among the feathers of its barn swallow host, where it feeds on keratin, mainly during the night (Marshall, 1981). We have never seen lice on tail and wing feathers of the host during daylight hours, when the host is active. The abundance of lice on particular hosts is consistent within and among years (Møller, 1991a). Previous studies have shown that male barn swallow hosts with long tails have a smaller abundance of this ectoparasite than short-tailed males (Møller, 1991a; Saino & Møller, 1994; Saino et al., 1995; Kose & Møller, 1999). Observational and experimental evidence suggests that testosterone levels in hosts affect the reproductive rate and hence the abundance of chewing lice (Saino & Møller, 1994; Saino et al., 1995). For example, the endocrinological state of hosts may affect lice through gland secretions that are applied to feathers. The chewing lice differentially feed on white

parts of feathers (Kose et al., 1999; Kose & Møller, 1999), apparently because the melanin content of this part is low. Habitat choice experiments have shown that there is a significant habitat preference for white parts of feathers (Kose et al., 1999). Foraging by chewing lice imposes serious costs on the host because feathers often break at sites where chewing lice have made holes (Kose & Møller, 1999), thus causing an increase in aerodynamic costs for the host. As barn swallow males with long tails have larger white tail spots than short-tailed males, we should expect that such hosts have more chewing lice. However, the contrary was the case (Kose et al., 1999; Kose & Møller, 1999), suggesting that long-tailed males could afford to have larger white tail spots than short-tailed males, as long-tailed males only suffered little damage to their white spots despite their large size.

The barn swallow is an approximately 20 g aerial insectivorous, semi-colonial passerine that feeds on the wing. The two sexes are very similar with respect to morphology with the exception of the outermost tail feathers which are on average 15–20% longer in males than in females in the Danish and Italian study populations (Møller, 1994a; N. Saino, unpublished data). Observations and experimental manipulation of tail length have demonstrated that long-tailed males experience several sexual selection advantages in terms of mating success, duration of the pre-mating period, differential parental investment by females, and paternity (review in Møller, 1994a; Møller & Tegelström, 1997; Saino et al., 1997a; Møller et al., 1998). Male and females share nest building and provisioning of nestlings, whereas only females incubate (Møller, 1994a). This secondary sexual character is condition-dependent with males in prime condition having long outermost tail feathers (Møller, 1989, 1994a; Møller & de Lope, 1994; Saino & Møller, 1996). Superior quality of long-tailed males is linked to absence of virulent parasites (Møller, 1990) and superior immune defence (Saino et al., 1995; N. Saino and A. P. Møller, unpublished data) that translate into a survival benefit for themselves and their offspring (Møller, 1991b, 1994b; Saino et al., 1997b).

Field procedures

We have followed two barn swallow populations, one at Kraghede (57°12′N, 10°00′E), Denmark 1988–2001 and a second one at Milan (45°28′N, 9°10′E), Italy 1993–2001. The study site at Kraghede consists of open farmland with pastures, cereals, potatoes and rape with mixed plantations, hedges and ponds. Barn swallows breed within barns and other buildings. See Møller (1994a) for further information. The study site at Milano consists of open farmland with pastures, cereals and hedges. Barn swallows are mainly restricted to dairy farms, where they breed inside buildings. Adult barn swallows were captured at their arrival from migration from late March to early April. Barn swallows were sexed from the shape of

their cloacal protuberance, sexual and breeding behaviour, and the presence (female) or the absence (male) of an incubation patch at first and subsequent captures. Individuals were marked with unique combinations of plastic colour rings on one tarso-metatarsus and markings on breast and belly feathers to allow identification of breeding pairs and assignment of adults to broods. A blood sample for parentage analyses was taken in heparinized capillary tubes by puncturing the ulnar vein. Offspring were individually marked when 6–13-days old, and subjected to sampling of blood for parentage assessment. When male offspring were captured as 1-year-old recruits, they were subjected to the same measurements as their parents by the same person.

We could confidently identify a bird as 1-year old when first captured as a breeder. Barn swallows from our study populations have very low breeding dispersal. This implies that individuals that have bred in a given colony in one breeding season do not move to another colony in subsequent years: In 10 study years in Italy, none of several thousand adult individuals ever moved to another colony to breed after having bred in a given farm. The same was the case for all but three of 3000 adults during 20 years of study in Denmark, and these three individuals then moved to the nearest neighbouring farm. We captured and marked more than 98% of all adult individuals in our study colonies (as determined by the number of unmarked birds at the end of the season). Hence, individuals that were captured for the first time as breeding adults could be considered at their first capture to have hatched in another farm during the previous breeding season unless already marked as nestlings.

Heritability estimates

Heritability was estimated for chewing lice using parent-offspring regressions for values when both parents and offspring were 1-year old. This standardizes parasite abundance for age effects that are known to exist for the chewing louse (Møller & de Lope, 1999). For the heritability analyses, we used a sample of 114 recruits from Denmark and a sample of 92 recruits from Italy from a similar number of nests recruited as breeders when 1-year old and the adults attending their nest when they also were 1-year old. We used parent-offspring regressions for fathers and sons only, because daughters disperse much further than sons (Møller, 1994a). As single parent-offspring regressions are sensitive to differences in variance among the sexes (Falconer & Mackay, 1996), we tested if variances differed. As we only had a single recruit from each nest, we could not make half-sib analyses. For the Italian sample we investigated parent-offspring regressions for within-pair and extra-pair offspring. We tested whether the relationship between louse abundance of offspring and parents differed between within-pair and extra-pair offspring with analysis of covariance with the number of lice of

offspring as the dependent variable, the number of lice of the father as the independent variable, and paternity as a factor. For all these analyses we used log $(x + 1)$-transformed values to obtain normal distributions, However we present untransformed values in the graphs for clarity of presentation.

Assessing the abundance of chewing lice

We assessed the abundance of chewing lice of the species *H. malleus* by carefully inspecting the tail feathers and the primaries and secondaries of the wings for the presence of holes chewed by this ectoparasite. This was performed by holding the feather up against a light source and counting the holes which typically have a diameter of 0.1–1.0 mm. Previous analyses have demonstrated that this procedure provides a highly repeatable measure of parasite abundance, with a repeatability of 0.99 (Møller, 1991a). Extraction of chewing lice from live birds, using an extraction jar with chloroform vapour, has shown that there is a strongly positive correlation between abundance of holes in the feathers and abundance of chewing lice (Pearson product-moment correlation coefficient $r = 0.62$, $n = 20$, $P < 0.01$ (Møller, 1991a)).

Estimating feeding rates

For a sample of 20 nests for which we had evidence of mixed paternity we assessed feeding rates of nestlings by marking nestlings individually on the breast feathers with permanent marker pens when nestlings were 12–15 days old. Nestlings were watched for 1 h daily observation sessions, and the total number of feeds per nestling per hour was subsequently calculated. This was done blindly with respect to paternity. After paternity had been determined (as described below), we calculated feeding rate for within-pair and extra-pair offspring for each nest.

Paternity analyses

We assessed paternity of the local recruits from Italy in order to be able to distinguish between additive genetic and common environment effects on the abundance of chewing lice on recruits and their presumed parents. Paternity was assessed by typing of four highly polymorphic microsatellite loci (*HrU3, HrU6, HrU9, HrU10*) isolated from the barn swallow (Primmer *et al.*, 1995; Primmer *et al.*, 1996) after Chelex extraction of DNA (Walsh *et al.*, 1991) and PCR amplification according to the procedures used in previous studies (Saino *et al.*, 1997b; Møller *et al.*, 1998). The average exclusion probability for these loci was 99.9% (Saino *et al.*, 1997b).

We considered an offspring to be sired by an extra-pair father whenever at least two of the genetic markers excluded the putative father as sire. This is a reliable method for paternity exclusion (Saino *et al.*, 1997b).

Fifty-five recruits from a similar number of nests turned out to be sired by the social mate of the female that laid the egg (within-pair offspring), whereas the remaining 37 recruits were sired by a male different from the social mate of their mother (extra-pair offspring).
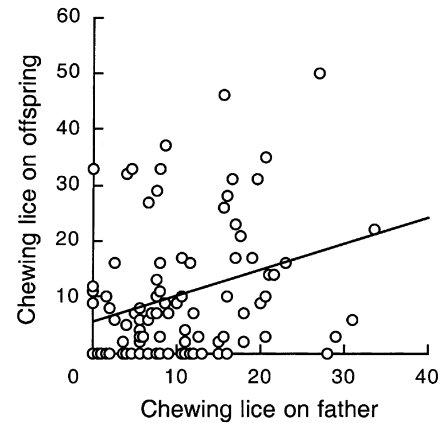
Results

The prevalences and the abundances of chewing lice in adult barn swallows from the two populations are reported in Table 1. Overall, there was no statistically significant sex difference in prevalence in either population (Denmark: $G = 0.001$, d.f. $= 1$, n.s.; Italy: $G = 0.045$, d.f. $= 1$, n.s.). The abundance of chewing lice was not normally distributed, whereas the log $(x + 1)$-transformed distribution of chewing lice did not differ significantly from normal (Lilliefors' test: n.s.). Therefore, all subsequent analyses are based on transformed data, although graphs present nontransformed data for clarity of presentation. There was no statistically significant sex difference in the mean abundance of chewing lice among adults (paired *t*-test for abundance on pair members based on log $(x + 1)$-transformed data: Denmark: $t = 0.72$, d.f. $= 113$, N. S.; Italy: $t = 0.13$, d.f. $= 91$, n.s.). Likewise, there was no significant difference in variance between males and females (*F*-max test: Denmark: $F_{113,113} = 1.18$, n.s.; Italy: $F_{91,91} = 1.05$, n.s.). There was no significant assortative mating with respect to chewing lice in the two study populations (Denmark: $r = 0.027$, $n = 114$, n.s.; Italy: $r = 0.089$, $n = 92$, n.s.). Recruits to the two study populations were strongly male-based because females disperse much further than males (Møller, 1994a), causing any separate tests based on daughters to have low power and therefore not be meaningful. Although there were no significant sex differences in prevalence and abundance of chewing lice, we were conservative by only analysing the abundance of chewing lice on sons and their father to avoid any heterogeneity because of offspring sex.

Offspring-father resemblance in abundance of chewing lice in the Danish population when both offspring and parents were 1-year old was statistically significant (Fig. 1). The linear regression based on the father–son regression was positive and statistically significant [$F_{1,112} = 21.01$, $r^2 = 0.157$, $P < 0.0001$, slope (SE) $= 0.39$ (0.09)]. Therefore, the heritability estimate was
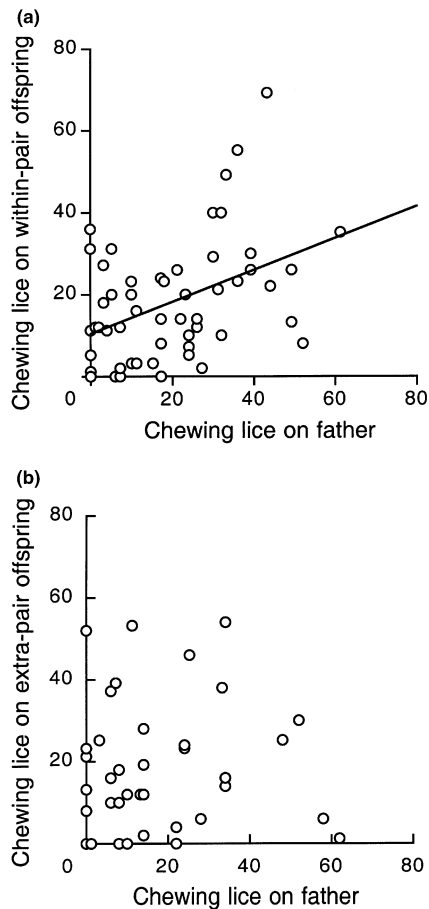

Fig. 1 Abundance of the chewing louse *Hirundoecus malleus* on offspring and fathers of their barn swallow host from Denmark. The regression line based on log $(x + 1)$-transformed data had the slope 0.39 (SE = 0.09). $n = 115$.

0.78 (SE = 0.18). This estimate may be biased, and we therefore investigated parent-offspring resemblance further by determining the paternity of offspring in another sample. The mother–son regression was also statistically significant [$F_{1,112} = 12.75$, $r^2 = 0.100$, $P < 0.001$, slope (SE) $= 0.35$ (0.09)].

In the Italian sample the heritability estimate for within-pair offspring based on the father–son regressions was 0.62 with a SE of 0.22 [Fig. 2a; $F_{1,53} = 7.81$, $r^2 = 0.128$, $P < 0.01$, slope (SE) $= 0.31$ (0.11)]. However, the regression for extra-pair offspring based on the father–son regression was not statistically significant [Fig. 2b; $F_{1,35} = 0.05$, $r^2 = 0.001$, n.s., slope (SE) $= 0.009$ (0.04)]. In fact, there was a statistically significant difference in the slope of the regressions for biological offspring and extra-pair offspring (overall ANCOVA model: $F_{3,89} = 5.62$, $P < 0.05$; paternity effect: $F_{1,89} = 6.49$, $P < 0.01$). The mother–son regression for the Italian sample was statistically significant [$F_{1,90} = 5.61$, $r^2 = 0.059$, $P < 0.05$, slope (SE) $= 0.18$ (0.08)].

Could the difference in resemblance for within-pair offspring-parents and extra-pair offspring-parents be due to differences in the phenotypes of the adults attending these two categories of offspring? When mothers of the two groups of recruits were compared, no significant differences were observed in morphology, abundance of three ectoparasitic arthropods (two species of chewing lice, including the one of the present paper, and a hippoboscid fly), measures of general condition (haematocrit, blood cell sedimentation rate, plasma protein concentration), or immunoglobulin levels (a measure of activation of the immune system which increases under parasitic infection) (*t*-tests; *t*-values always n.s.) [see Saino *et al.* (1999) for methods]. More specifically for *H. malleus*, there was no statistically significant difference in the abundance of lice between females with extra-pair

Table 1 Prevalence (%) and abundance of chewing lice in adult male and female barn swallows.

	Prevalence (%)	Mean	Variance	Median	n
Denmark					
Males	79.8	11.21	95.73	8.0	114
Females	79.8	11.48	108.30	9.0	114
Italy					
Males	85.7	18.82	265.67	9.5	92
Females	89.1	19.17	372.70	15.0	92

Fig. 2 Abundance of the chewing louse *Hirundoecus malleus* on offspring and fathers of their barn swallow host from Italy. (a) Within-pair offspring. $n = 55$. The regression line based on log $(x + 1)$-transformed data had the slope 0.31 (SE = 0.11). (b) Extra-pair offspring $n = 37$. The regression line based on log $(x + 1)$-transformed data was not significant and had the slope 0.01 (SE = 0.17).

offspring and females with intra-pair offspring (ANOVA based on log $(x + 1)$-transformed data: $F_{1,90} = 0.04$, n.s.). Furthermore, the mother–offspring regressions for these two categories of offspring had large slopes that did not differ significantly from each other (overall ANCOVA model: $F_{3,89} = 5.38$, $P < 0.05$; paternity effect: $F_{1,89} = 0.06$, n.s.). Hence, no evidence suggested that mothers of within-pair sons differed from those of extra-pair sons.

Finally, offspring may differ in susceptibility to parasites because extra-pair offspring receive a different amount of food than their within-pair siblings. We investigated in another study whether rate of parental care to offspring differed between within-pair and extra-pair offspring in the same broods. Feeding rates to nestlings (feedings per nestling per hour) did not differ significantly between the two categories of nestlings [mean feeding rate per nestling per hour (SE): within-pair offspring: 2.29 (0.13), extra-pair offspring: 2.40 (0.17); paired *t*-test, $t = 0.91$, d.f. = 19, n.s.] (N. Saino, M. Incagli and A. P. Møller, unpublished data).

Discussion

Adult male and female barn swallows have uniformly high prevalences and abundances of chewing lice of the species *H. malleus* (Table 1). Offspring consistently showed similarity in parasite abundance with their parents (Figs 1 and 2). We attempted to distinguish between additive genetic and environmental determinants of resemblance of the abundance of parasites in fathers and offspring by exploiting the fact that approximately a third of all offspring are extra-pair offspring (Møller & Tegelström, 1997; Saino *et al.*, 1997b; Møller *et al.*, 1998). This creates two groups of offspring that share a common environment, but differ genetically because they are half-siblings. Hence, we can use resemblance for extra-pair offspring and for within-pair offspring to distinguish between the hypotheses that resemblance is caused by additive genetic variation and by a common environment and maternal effects. When we compared resemblance between fathers and extra-pair and within-pair offspring, we found a significant difference (Fig. 2a,b). The resemblance for within-pair offspring was significant and positive, whereas the value for extra-pair offspring was weak and nonsignificant. Furthermore, the degree of resemblance differed significantly between the two groups of offspring. There was no bias in these estimates because of assortative mating (Falconer & Mackay, 1996) as males did not significantly resemble their partners with respect to abundance of chewing lice. This suggests that resemblance in parasite abundance between fathers and sons is caused by additive genetic variance. This is despite the fact that this parasite is a directly transmitted ectoparasite. The method adopted for assessing the relative importance of additive genetic variation and common environment as determinants of phenotypic values has potentially broad application (see Merilä & Sheldon, 2001). That is particularly the case given the fact that females of many animals regularly copulate with more than a single male during a single fertile period, and given that paternity analyses are now commonplace (Birkhead & Møller, 1998). The difference in heritability estimate for extra-pair and within-pair offspring was large and statistically significant. This finding differs from the previous estimates based on extra-pair and within-pair offspring, which were all relatively similar (Merilä & Sheldon, 2001). It remains unknown why that should be the case, and many more estimates for different characters will be needed before a possible hypothesis that accounts for this difference can be proposed.

Our conclusions about genetic variation in parasite abundance can only be accepted if alternative explana-

tions can be ruled out. Which potentially confounding effects could have generated a positive association between the phenotype of male offspring and that of their biological fathers, but not that of their foster fathers? The most obvious answer is that the mothers of the two groups of recruits differed consistently in phenotypic traits such as morphology, rate of parasitism or condition. However, the mothers of extra-pair offspring recruited into the breeding population were not significantly different in terms of morphology, physiology and parasite load from mothers of within-pair offspring. Specifically, the abundance of *H. malleus* did not differ significantly between mother of extra-pair and within-pair offspring. For the mother-offspring regression there was no significant difference in resemblance for within-pair and extra-pair offspring. This suggests that substantial maternal effects could not cause the difference in resemblance between males and within-pair and extra-pair offspring, respectively. Similarly, parents were apparently unable to discriminate between within-pair and extra-pair offspring as the two categories of offspring received food at a rate that did not differ significantly. Thus, differential feeding of extra-pair offspring could not account for the differences found. Finally, parents do not preen their offspring, so individual differences in preening activity cannot account for the findings.

Although additive genetic variance accounted for some of the resemblance in phenotype between parents and offspring, the relative importance of different environmental components of variance remains undetermined. If common environment effects were strong, we should expect that parents resembled each other in terms of lice abundance. That was not the case. Hence, we can conclude that there is little evidence of common environment effects. There is more scope for an effect of maternal effects as we have shown that maternal immune components are transferred to eggs and nestlings, and that such maternal transfer has an impact on parasite-mediated natural selection (Saino *et al.*, 2001, 2002a,c).

Female barn swallows are known to differential feed their offspring when mated to an attractive male (de Lope & Møller, 1993; Møller, 1994c; Saino & Møller, 1995). However, such differential parental investment by females cannot have affected the resemblance between parasite loads of offspring and that of their fathers. Male tail length is only known to affect the relative feeding effort of parent barn swallows, but not the total amount of food provisioning by the breeding pair (de Lope & Møller, 1993; Møller, 1994c; Saino & Møller, 1995).

We can only speculate about the mechanisms generating additive genetic differences in parasite load. For the chewing louse it is possible that host differences in feather quality or secretions from the preen gland make certain hosts more susceptible to infestation than others.

Parent–offspring regressions based on extra-pair and within-pair offspring revealed that similarity in chewing louse abundance between relatives was caused by shared genes rather than a shared environment, despite the fact that these are directly transmitted ectoparasites.

Acknowledgments

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