Parasites during early life mediate the strength of phenotypic selection on sexual traits.

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Abstract

Sexually selected phenotypes are key to understanding population and eco-evolutionary dynamics since the expression of these traits is tightly linked to reproduction and population viability. Parasites are major agents of selection that alter both phenotypic expression and fitness. However, the carry-over effects of parasites in early life in selection on phenotypes expressed later in life are unknown. We capitalised on a long-term monitored population of pied flycatchers, *Ficedula hypoleuca*, where nestlings are naturally exposed to nest-dwelling, blood-sucking blowfly larvae. We explored whether parasitism by blowflies during early life and breeding mediates selection on forehead patch size (FPS), a sexual and social signal in the study species. We also explored whether FPS of males is a reliable proxy of ectoparasite burden of their offspring. We found that the strength of selection on FPS increased with the abundance of blowfly larvae only during growth, but also, that male FPS was a reliable predictor of the blowflies burden of their offspring. We highlight the major role of parasites as agents of selection in early life, but not during reproduction in adulthood. We discuss the implications of our results on the direct and indirect benefits females may obtain by mating to highly ornamented males.

Introduction

Sexually selected phenotypes are key actors in population and eco-evolutionary dynamics due to their tight link to reproduction and population viability [1]. Among the multiple factors that shape sexual selection, parasites are one of the most relevant agents of selection that can determine the expression of sexual traits and their evolutionary trajectories [2, 3] by mediating the trade-off between trait expression and individual quality [4-6]. Early-life conditions can have a carry-over influence on the resolution of life-history trade-offs that may ultimately drive fitness. Environmental conditions during early development can have a deep impact on fitness components later in life [7, 8], life-history strategies [9] as well as on phenotypic expression, including that of sexual traits [10-12]. However, the influence of parasite burden in early life on the eco-evolutionary dynamics of sexual traits in adulthood is an unexplored question.

In evolutionary terms, selection should favour high-quality individuals showing enhanced expression of their ornaments [13, 14]. When considering parasites, the immunocompetence handicap principle (ICHH) suggests that only highly ornamented individuals are able to withstand the costs imposed by parasites [15]. The ICHH proposes that more ornamented males may provide indirect (genetic) benefits to females by improving offspring ability to avoid, tolerate or resist parasite infection [2]. Such indirect (genetic) benefits influence individual capacity to resist or avoid parasite infection during early development, having a potential impact on the production or maintenance of sexual traits later in life [15]. Therefore, parasites may also be a key selective agent acting during growth, thus mediating phenotypic selection of sexual traits later in life. However, phenotypic selection, understood as the covariation between the expression of a trait and fitness, is not always constant but may fluctuate due to variation in the strength of the agents of selection [16]. In general terms, selection on phenotypes is expected to increase as environmental conditions worsen [16-19]. Similarly, high parasite loads reduce the evolutionary potential of morphological traits of the host [3] and increase condition-dependence of sexual traits [6]. Despite such evidence, no formal test has been conducted to quantify the mediating role of parasite burden during ontogeny in selection on sexual traits.

In addition to the potential indirect benefits accrued to females, direct models of sexual selection also support the role of non-genetic, direct benefits to females when paired with more ornamented males. There is ample evidence that males displaying more conspicuous traits provide direct benefits to females, such as increased fertility or more parental care [20, 21]. In addition, mating with males with low parasite burdens may reduce the parasite load of the females [22], suggesting that choosing less parasitized mates also provides direct benefits to females. If parasite avoidance or resistance can be considered as a direct benefit, it would be expected that elaborated ornaments in males can be used as predictors of the parasite load or exposure for their descendants, which may directly increase own female fitness.

The unifying goal of this study is to explore the carry-over effects of blood-sucking ectoparasites on the strength of phenotypic selection on sexual traits. To achieve this aim, we tackle two interconnected questions. First, we explored the mediating role that parasites in early life may have as drivers of selection on the expression of a sexual signal at adulthood. Second, we explored whether the expression of a sexual trait at adulthood can act as a reliable indicator for females of the parasite burden of their offspring. To answer these questions, we benefited from a long-term data set collected over 31 years in a population of pied flycatchers (*Ficedula hypoleuca*) located in central Spain, including individual information on the reproduction and sexual trait expression of 994 males. We focused on the effects of *Protocalliphora* blowflies, whose larvae are obligate blood-sucking nest ectoparasites of nestling birds [23], on the forehead patch size (FPS) of males, a model trait in sexual selection studies [24]. The FPS is a sexual and social signal in pied flycatchers [25, 26], as males with large forehead patches enjoy competitive [25, 27] and mating advantages [26]. Blowfly larvae have adverse effects on nestling fitness and body condition in early life [28-37] and can also have a carry-over effect reducing female fitness [38]. According to our two main objectives, we first hypothesize a mediating role of these parasites during growth in selection on the expression of male FPS later in life. We predict an increase in selection as parasite burden raise. Secondly, we predict that FPS at adulthood is a reliable predictor of ectoparasite infection in their breeding nests.

Material and Methods

Population monitoring

Data were collected between 1989 and 2021 during a long-term study of pied flycatchers breeding in nest-boxes located in central Spain (La Hiruela, 41°04' N, 3°27' W). Because of limited field effort in 2002 and 2003, these years were excluded from analyses [9, 39]. The population occupies an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a mixed coniferous plantation dominated by *Pinus sylvestris* located 1.1 km apart [see 40 for a description of the study area]. Breeding seasons last from the third week of April to the first fortnight of July. Nest-boxes (n=237) were inspected every 2-3 days to record laying date (date of the first laid egg), clutch size, number of hatchlings, and number of fledglings. Breeding individuals were captured using a nest-box trap, individually marked with coloured (only males) and metal (males and females) rings, measured for tarsus length (±0.05 mm), weighed (±0.1 g), and aged as either one-year old or older following the criteria of Karlsson et al. [41]. FPS of males was measured with a digital calliper (± 0.01 mm) as height × width of the smallest enclosing rectangle [42, 43]. Using the package rptR v. 0.9.22 [44], the adjusted repeatability of FPS corrected by age for 942 males and 1756 observations was 0.712 ± 0.018 (Confidence intervals: 0.674 - 0.745).

All nestlings were ringed at the age of 13 days with metal rings. This population of pied flycatchers shows high breeding site fidelity (96% return to the patch of first reproduction) and local recruitment rates (around 13%) are among the highest reported for the species [45, 46], suggesting that most fledglings that survive to breeding age return to our nest-box areas. Unringed adults, defined as immigrants, represent about 30% of the population, and their proportion in relation to population density has been constant over time (unpubl. data). We are confident that recaptures of offspring hatched and breeding in the area are unbiased for several reasons: i) we capture nearly all adults in the population [47]; ii) there is no familial resemblance in dispersal patterns, meaning that the offspring of dispersers are not more prone to disperse than those of residents [40]; iii) breeding outside the study plots, either in the surroundings (as indicated by surveys conducted during the breeding seasons; pers. obs.) or in more distant areas, including other study populations of Iberian flycatchers (as indicated by ring recoveries), is an extremely rare event (pers. obs.) [46]; iv) mark-recapture analyses have shown that variation in the probabilities of survival (transition) and recapture do not vary over time [39].

Blowfly species and quantification

Blowfly (*Protocalliphora azurea*) females oviposit in the nests in spring. This species' larvae inhabit nests and are obligatory nest-dwelling ectoparasites. They start to develop after nestlings hatch [23] by attaching to them and feeding intermittently on nestling blood before burrowing through the nest material to pupate [48]. The newly emerged adults spend the winter in diapause until spring, when the females deposit eggs in bird nests [23].

We used the number of pupae counted in the nest to quantify blowflies. Blowfly numbers were assessed by carefully disassembling the nest contents shortly after the young fledged and noting the presence or absence of blowfly larvae and/or pupae buried in the nest material. We consider prevalence as presence/absence of blowflies, and abundance as the number of blowfly pupal cases in the nest [including zero – 49]. We are fully confident that all the pupae we found in the nests belonged to the species *Protocalliphora azurea*, since no other species has been found among the adults emerged (in lab conditions) from more than 50 nests examined in different years [38, 50].

Statistics

We quantified the mediating role of parasites in selection on male FPS. We fitted two sets of Generalized Linear Mixed Models (GLMMs) using the package glmmTMB v.1.0.2.9000 [51] to test whether parasites mediate selection on FPS during two different life-stages, early-life (*t*) and adulthood (*t+1*). We run the models for each life-stage considering number of fledglings or number of recruits as fitness proxies, fitted with a negative binomial or Poisson distribution, respectively. As explanatory term, we z-transformed FPS (zFPS) within each year to a mean of 0 and SD of 1 to control for the environmental covariance between fitness and the trait value across years [17, 52, 53].

To test the mediating effect of blowflies during early life (*t*) in selection on FPS at adulthood (*t+1*), we considered the interaction between zFPS (*t+1*) and either prevalence (0: absence; 1: presence) or abundance (number of larvae/pupae) of blowflies in the natal nest of the male (Blowflies$_t$: blowflies during growth) as explanatory terms in the four corresponding models. Male age, habitat, and laying date were also included as explanatory variables. Male age was considered to account for age-related differences in breeding performance [54, 55] and coded as 1-year-old or older, since the exact age for immigrants can only be assigned to these two categories. Habitat was coded as a two-level factor (oak or pine) to control for consistent differences in mean fitness between habitats [40]. Finally, we included breeding (egg laying) date to account for seasonal effects on fitness [16, 56]. We used individual identity and year as random terms to control for repeated measures of the same individuals across years.

To quantify the mediating effect of parasites in selection on FPS during reproduction, we repeated the models above using the prevalence or abundance of blowflies in the nest during breeding (Blowflies$_{t+1}$; blowflies during reproduction). We also repeated the analyses restricting the data set to males in which ectoparasite data were known during growth. In these analyses, the abundance or prevalence of blowflies as covariates in different models.

To explore whether FPS was a reliable or honest proxy for the prevalence or abundance of blowflies in the nest, we considered FPS as dependent variable in a linear mixed model [57, 58], using the package lme4 v. 1.1-26 [59]. Prevalence or abundance of blowflies was included as an explanatory term in the different models, along with male age and habitat as explanatory terms. We used individual identity and year as random terms to control for repeated measures of the same individual in different years. All models were run in R v. 4.0.4. [60].

Results

Mean abundance and prevalence of blowflies across years were 3.3 ± 0.1 (SE) and 0.4 ± 0.1 (SE) per nest, respectively. We determined the prevalence and abundance of blowflies experienced during growth (t) in 592 breeding males, from which we quantified their number of fledglings and recruits. In addition, information on the prevalence and abundance of blowflies in the nests at adulthood ($t+1$) was available for 658 males, from which we quantified their number of offspring and recruits.

Regarding the ontogenetic effects of blowflies in selection on FPS at adulthood, we found that selection on FPS was mediated by the abundance of blowflies during growth (Blowflies$_t$ - Table 1). Specifically, using the number of recruits as a fitness proxy, the selection gradient increased with the abundance of blowflies (Figure 1). However, the blowfly prevalence did not mediate selection on FPS (Table 1), neither using the number of fledglings or recruits as fitness proxies (Table 1).

During breeding, abundance of blowflies$_{t+1}$ did not mediate selection on FPS neither considering number of offspring (interaction FPS x blowflies$_{t+1}$; estimate: -0.001±0.002, z=-0.565, p=0.572) nor number of recruits (interaction FPS x blowflies$_{t+1}$; estimate: -0.004±0.008, z=-0.510, p=0.610). None of these results changed when restricting our analyses to those males we knew their blowfly burden during growth (interaction FPS x blowflies$_{t+1}$ for the number of offspring or recruits, p=0.208 and p=0.445, respectively). Lack of statistical significance held when prevalence of blowflies was considered using number of recruits (interaction FPS x blowflies$_{t+1}$; estimate: 0.117±0.086, z=1.372, p=0.170) or number of offspring as fitness proxies (interaction FPS x blowflies$_{t+1}$; estimate: -0.006±0.030, z=-0.208, p=0.835). The results did not change using the reduced data set (interaction FPS x blowflies$_{t+1}$ for the number of offspring or recruits, p =0.583 and p=0.255, respectively).

FPS of males was a reliable proxy of abundance (estimate: -0.1539±0.0769, t=-2.000, p=0.045) and prevalence (Estimate: -3.4248±0.1964, t=-3.551, p<0.001 – Figure 2) of blowflies in their breeding nests.

Discussion

Our results suggest that parasites during growth mediate selection on a secondary sexual trait of the host at adulthood. Specifically, we described that the strength of selection on ornament size increases with the abundance of parasites during early life. However, parasites do not influence selection on the ornament during breeding. These findings highlight the deferring role of parasites in selection on sexual traits during growth but not during reproduction, stressing the stronger effect of parasites as agents of selection in early life. In addition, the size of the male ornament is revealed as a good indicator of the prevalence and abundance of ectoparasites of their offspring in their breeding nests. Overall, our results point out the knock-on effect that parasites during early life have on the strength of selection on sexual traits displayed in later life stages.

To the best of our knowledge, this is the first time that the strength and direction of selection that parasites impose on the sexual traits of their host has been quantified in two different life stages. Our results highlight the role that an agent of selection have on selection of sexual traits, not at the time of breeding, but in early life, suggesting that the strength of selection imposed on FPS is not uniform along an individual life-cycle. The role of parasites on sexual selection has been described theoretically [2, 15] and proved experimentally [61-64], but the evidence of parasites in shaping selection at the phenotypic and genetic level is rather limited. We stress the crucial role of this agent of selection during early life in shaping the evolutionary trajectory of a sexual trait, at least at the phenotypic level.

From an evolutionary perspective, studies in wild populations exploring the environmental factors that shape the evolutionary trajectory of secondary sexual traits are limited and mainly focused on climatic factors [1, 65]. Even within this biased evidence, conclusions are far from consistent [reviewed in 65]. This lack of agreement can be resumed in two recent studies where climate upends selection on sexual traits at the genetic level in collared flycatchers [66] and a lack of evolutionary change in blue tits [67]. These differences could be explained by multiple factors, including differences in the biology of species or the physiological bases of production of the sexual trait considered (melanin- versus carotenoid-pigmented or structural traits, for example). Also, climatic factors cannot be strictly considered agents of selection but rather environmental drivers that may covary with biotic or abiotic factors that act as the ultimate agents of selection [68]. Previous studies suggest that heterogeneity of environmental conditions can influence selection at the phenotypic level on life-history traits [16-18]. Our study identifies the environmental factors that modulate selection, and points towards a specific agent of selection. Under selection in poor environmental conditions, it is expected that only high-quality individuals can withstand the cost of producing or maintaining a sexual signal without a fitness payoff. Our results agree with this idea, where highly ornamented individuals are only favoured in reproductive terms when exposed to high parasite burdens during early life. Following this rationale, under favourable environmental conditions, the differential benefit of showing a sexual trait for high- and low-quality individuals vanishes, perhaps because all individuals can cope with the fitness costs associated with expressing a more elaborated ornament.

By definition, parasites impose costs on hosts that have effects, among others, at the genetic [69-71], physiological [72-75], behavioural [61, 76-78] and demographic levels [79-81]. However, the specific mechanism through which parasite infection during early life shapes the covariation between fitness and ornamental expression in adulthood is unknown and out of the scope of this study. Certainly, more intensively parasitized individuals may be expected to have reduced condition and health, leading to decreased survival and/or reducing breeding performance among survivors [7, 8, 82, but see 83]. This is also the case for blowfly larvae nest parasitism, where chicks experience multiple short-term detrimental effects, such as an increased risk of mortality and impaired growth or physiology [28-37, 84]. Blowflies may also cause long-lasting effects on reproduction, with parasitized females as nestlings laying smaller eggs throughout their lifetime [38]. We propose that the impact of parasites on host health during early life has a carry-over effect at the time of breeding, particularly when parasite infection is high [82]. From a mechanistic point of view, the key issue is determining what causes such health deterioration. We can speculate that the pathogens of the genus *Mycobacterium* transmitted to blowfly-infected nestlings [85, 86] or increased begging of parasitized broods [87] could be mechanisms explaining the effect of parasites on selection later in life. We support the view that under the ICHH [2], only high-quality individuals showing an enhanced version of the ornament will be able to withstand the costs of being parasitized during development. Expanding on this idea, it is plausible that the costs of producing an elevated immune response against parasites during growth can lead to a carry-over effect on health later in life [82], that only high-quality individuals can afford. Therefore, only high-quality individuals that experienced high parasite abundance during early life could stand a positive covariation between fitness and the expression of a sexual trait, thus being positively selected.

We also found that the size of sexual traits in breeding males was a reliable proxy of the nest-dwelling ectoparasite load in their breeding nests and, therefore, their offspring. Specifically, offspring of males with an enhanced expression of the forehead patch had lower rates of parasite infection. Therefore, females mating with males expressing large ornaments would benefit from raising chicks with a low probability of infection during the nestling stage. Further, females could also avoid their own infection by reducing exposure to parasitism. The combined result of i) stronger selection on ornament size when parasite abundance is high during early life and ii) the reliability of trait size as a predictor of parasite burden in the offspring has several implications within a sexual selection context. Two main models have been proposed to understand the evolution and maintenance of the expression of sexual traits. On the one hand, direct models arise when females improve their own fertility or viability by mating to highly-ornamented males [88]. On the other hand, indirect models arise when females obtain genetic benefits for their descendants by choosing the most ornamented males [89, 90]. These two models are usually confronted despite there is scope for integrating both direct and indirect models to explain the evolution of sexual traits [91], albeit evidence is scarce in wild conditions [but see as example 92]. Our results support the prediction of indirect models of sexual selection, as expressing a superior ornament in males may signal their capacity to stand high parasite loads during growth without compromising future fecundity. Also, since the expression of the sexual trait is a reliable predictor of the parasite burden of nest (t+1), our results are partially in agreement with direct models of sexual selection since it claims a direct female fitness benefit, measured as reduced parasite infection. Interestingly, another line of support for direct models of sexual selection may stem from the assumption that individuals that experience poor environmental conditions during growth are able to hold a high-quality habitat during breeding [93], meaning a direct benefit for choosy females. Our study, however, was not designed to disentangle the fitness benefits of choosing a highly ornamented male that accrue to females or offspring in terms of reduced parasite infection. Still, we argue that, along with other potential benefits, low levels of parasite infection may reduce the costs that blowflies may have on breeding females as well.

In conclusion, our results suggest that the abundance of a nest-dwelling ectoparasite in early life mediates selection on a male sexual ornament at adulthood by increasing the intensity of positive directional selection when parasite abundance is higher. Interestingly, we described that the intensity of selection on a sexual traits of this parasite differs in two different life-stages, being stronger during growth than during adulthood. Further, the expression of this sexual trait is a reliable predictor of parasite burden in the breeding nest and, therefore, the offspring. Our results support broadening the ICHH to early life, where parasites may impose a higher cost during growth than during breeding. It can be postulated that only high-quality individuals during early life are able to confront high parasite abundance without compromising the covariation between the expression of a sexual trait and fitness. Within a sexual selection context, our results highlight the direct and indirect genetic benefits that females may gain by mating with highly ornamented males, both by increasing female fitness and by ensuring a high genetic quality of descendants to cope with parasite infection.

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Table 1. Results of GLMMs testing for the interactive influence of abundance and prevalence of blowflies$_t$ (during growth) in selection on FPS, considering the number of recruits or number of fledglings as proxies of fitness.

	Abundance of blowflies				Prevalence of blowflies		
	Variable	*Estimate [±SD]*	*z*	*p*	*Estimate [±SD]*	*z*	*p*
Number of recruits	(Intercept)	-1.4731 [0.3032]	-4.857	<0.001	-1.42863 [0.2953]	-4.836	<0.001
	Laying date	-0.0120 [0.0064]	-1.861	0.062	0.0133 [0.0061]	-2.176	0.029
	Male age	0.4075 [0.1318]	3.092	0.002	0.4065 [0.1263]	3.218	0.001
	FPS	-0.0730 [0.0504]	-1.448	0.147	-0.0421 [0.0561]	-0.751	0.452
	Blowflies	0.0039 [0.0067]	0.582	0.560	-0.0593 [0.0908]	-0.653	0.513
	Habitat	0.5237 [0.0935]	5.600	<0.001	0.4796 [0.0892]	5.372	<0.001
	FPS*blowflies	**0.0143 [0.0068]**	**2.091**	**0.036**	0.057 [0.0915]	0.623	0.533
Number of fledglings	(Intercept)	0.7690 [0.0998]	7.704	<0.001	0.7751 [0.0960]	8.069	<0.001
	Laying date	0.0048 [0.0020]	2.412	0.016	0.0033 [0.0019]	1.758	0.078
	Male age	0.2918 [0.0438]	6.654	<0.001	0.3024 [0.0418]	7.233	<0.001
	FPS	0.0048 [0.0168]	0.289	0.772	0.0048 [0.0188]	0.255	0.7991
	Blowflies	0.0011 [0.0022]	0.498	0.618	-0.0047 [0.0300]	-0.157	0.875
	Habitat	-0.0129 [0.0318]	-0.405	0.685	-0.0173 [0.0301]	-0.575	0.565
	FPS*blowflies	0.0002 [0.0024]	0.103	0.917	-0.0061 [0.0302]	-0.205	0.837

Figure 1. Mediating role of the abundance of blowflies$_t$ during growth in selection on FPS. For graphical purposes, we split blowflies abundances into three groups to illustrate how the selection gradient, calculated as the covariance between FPS and number of recruits, changes in relation to parasites during growth. In this case, abundance of blowflies was categorized at unequal 3 levels (low: 0 - <2 blowflies; intermediate: ≥2 - <10; high: ≥10) to visualize the difference in selection gradients. Note that selection in the "low" category is fixed to 0 and therefore used as the reference level for the rest of selection gradients. Sample size is shown for each group. The formal test and results are given in the main text.

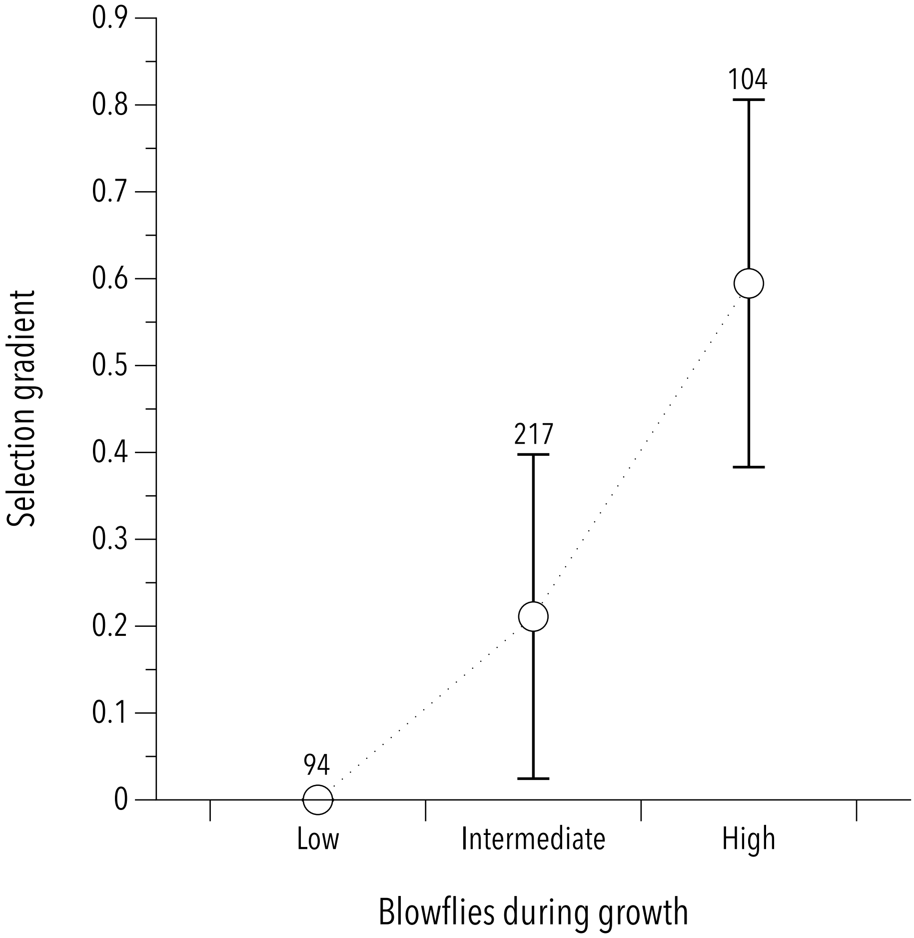

Figure 2. Difference in FPS of male pied flycatchers in relation to the prevalence of blowflies found in their breeding nests. Number of males is shown for each group. Note that sample size differs from the one given in the text since the same male may have difference prevalence throughout his life.