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Fitness outcomes in relation to individual variation in constitutive innate immune function

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Although crucial for host survival when facing persistent parasite pressure, costly immune functions will inevitably compete for resources with other energetically expensive traits such as reproduction. Optimizing, but not necessarily maximizing, immune function might therefore provide net benefit to overall host fitness. Evidence for associations between fitness and immune function is relatively rare, limiting our potential to understand ultimate fitness costs of immune investment. Here, we assess how measures of constitutive immune function (haptoglobin, natural antibodies, complement activity) relate to subsequent fitness outcomes (survival, reproductive success, dominance acquisition) in a wild passerine (*Malurus coronatus*). Surprisingly, survival probability was not positively linearly predicted by any immune index. Instead, both low and high values of complement activity (quadratic effect) were associated with higher survival, suggesting that different immune investment strategies might reflect a dynamic disease environment. Positive linear relationships between immune indices and reproductive success suggest that individual heterogeneity overrides potential resource reallocation trade-offs within individuals. Controlling for body condition (size-adjusted body mass) and chronic stress (heterophil-lymphocyte ratio) did not alter our findings in a sample subset with available data. Overall, our results suggest that constitutive immune components have limited net costs for fitness and that variation in immune maintenance relates to individual differences more closely.

1. Introduction

Immune defences are crucial for the health and survival of host organisms among a diversity of parasites, pathogens and diseases. Considering this importance, any individual variation in host immune function is likely to be consequential for defence against disease-causing agents and host survival and longevity [1]. Additionally, many parasites and pathogens have sub-lethal effects [2] that either directly or indirectly influence host fitness. Infections and diseases can disrupt the host's reproductive pathways [3,4], the ability to acquire resources [5,6], ornamentation and the signalling of attractiveness to a mate [7,8], or even social dominance or prestige [9,10]. Parasites can therefore affect many fitness-related traits that will ultimately have consequences for the overall fitness of the host. For hosts, investment into diverse and multi-faceted immune systems to abate parasite pressure should therefore relate to overall fitness [11].

Operating an immune system is physiologically and energetically costly, incurring development, maintenance and activation costs, in addition to any collateral damage sustained through immune-associated inflammation [12,13]. Consequently, when resources are limiting, trade-offs are expected to occur between immune function and other key physiologically demanding processes [14,15]. Maintaining immune function at a sub-maximal level might therefore be the optimal solution for hosts to maximize overall fitness [16–19], and as a

result tolerance to parasites can emerge [20,21]. Although there is some uncertainty over when and how the costs of immune function are paid physiologically [14,22,23], there is good evidence that physiological trade-offs do exist, e.g. between immune function and reproduction [24–26], growth [27] and moulting [28,29]. Experimentally manipulated resource availability has also demonstrated that different physiological processes compete for resources on some level [26,29], and it remains possible that energy is not the only currency of these trade-offs [22]. Despite attempts to quantify the absolute and relative physiological costs of immune function [13,14,23], relatively little is known about how variation in individual immune investment ultimately relates to fitness or important fitness-related traits.

When linking the proximate physiological costs to the ultimate fitness costs of immune function, it is particularly important to examine fitness in the wild, under natural environmental constraints and associated trade-offs [30,31]. Moreover, because immune systems coevolved with diverse parasite pressures and mediating environmental stressors [32] that are often removed in controlled conditions, immune function can greatly differ in wild versus captive conditions [33]. However, whether in wild or captive conditions, the costs associated with activating (*induced*) immune responses have been widely investigated as immune activation is relatively tractable using experimental immune challenges [34,35]. Consequently, there is substantial evidence that immune activation is physiologically costly, resulting in trade-offs with fitness-related traits, particularly reproductive effort and success (reviewed for avian species by [22]). As meaningful experimental manipulation of baseline (*constitutive*) immunity is considerably more difficult, however [14], the costs of constitutive immune function have received much less attention (but see [36–38]). Although presumably lower than activation costs [13,23], constitutive costs do accrue continuously and might only cumulatively translate into fitness costs. However, how individual constitutive immune variation relates to fitness consequences in the wild has not been rigorously tested (but see [39–41]).

In this study, we assess how individual variation in constitutive immune function is related to subsequent fitness outcomes (survival, reproductive success, dominance acquisition) in a wild, cooperatively breeding, tropical passerine, the purple-crowned fairy-wren. We measure three immune indices frequently measured in wild animals and known to be important for front-line defences (haptoglobin (Hp), natural antibodies (NABs), complement activity (CA); [42–44]). Although these indices could partly vary as a result of the unknown disease environment of wild animals, they are constitutively maintained and vary among individuals even in the absence of infection [45,46]. Using this approach, we implicitly interpret fitness outcomes as a subsequent consequence of individual variation in the maintenance of immune function. Given the importance of longevity for purple-crowned fairy-wrens, we predict that individuals with higher levels of the constitutive immune indices will have higher survival probability. Congruent with evidence of resource reallocation trade-offs between reproduction and immune activation [22,47], we predict that higher levels of constitutive immune indices (immune system maintenance) will be associated with reduced subsequent reproductive success. Lastly, we hypothesize that the acquisition of a dominant breeding position is a function of

individual quality and/or ability to sequester and efficiently use resources. Therefore, we expect that higher levels of the immune indices will predict acquisition of a dominant breeding position.

2. Methods

(a) Study species

Purple-crowned fairy-wrens (*Malurus coronatus coronatus*) are cooperatively breeding residents of small rivers in northwest Australia. As riparian specialists, they are highly dependent on *Pandanus aquaticus* vegetation for nesting, foraging and predator evasion [48]. Social groups of 2–11 individuals defend stable year-round territories. A dominant male and female are the only birds in each group to breed, while commonly one or more subordinate adults (settled dispersers or previous offspring) contribute to offspring provisioning [49,50]. The dominant pair are socially and genetically monogamous, with extra-pair paternity (4%) used primarily as a means of incest avoidance [51]. Breeding occurs after rainfall at any time, peaking annually during the wet season (December–March; [52]). The core study population is located in Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.1° E, –17.5° N), where every individual along a contiguous 15 km of Annie Creek and Adcock River has been uniquely colour-banded since 2005. Territory boundaries, social group composition, individual movements and dispersal within the population and survival are monitored through regular censuses. In this tropical riparian habitat, purple-crowned fairy-wrens are exposed to a rich variety of parasites with recorded infections by *Plasmodium* spp. and *Haemoproteus* sp. of avian malaria, *Myrsi-dea* sp. of lice, *Coccidia* spp., *Trypanosoma* sp., Hippoboscids flies and microfilarial nematodes [53,54]; infection prevalences for these parasites appear relatively low, but could vary temporally and/or spatially with the availability of standing water.

(b) Capture and sampling

Capture, sampling and population census were done on a biannual basis, during two fieldwork sampling and census periods from mid-April to mid-June, and from mid-October to late November each year, at the start and end of the dry season, respectively (hereafter 'fieldwork seasons' in either 'May' or 'November'; electronic supplementary material, figure S1). For this study, 716 captures of 351 adult (greater than 90 days old) purple-crowned fairy-wrens were made during 11 consecutive fieldwork seasons in both May and November from May 2012 to May 2017 (mean = 119 samples collected per year, s.d. = 49; May only in 2017). Of the 351 sampled individuals, 181 were male and 170 were female, with some repeated measures per individual (median = 2, range = 1–7; one sample per individual per fieldwork season). Fairy-wrens were caught in mist-nets and held in bags until blood sampling (median = 23 min, s.d. = 19.6 min). Up to 100 µl of blood was collected into heparinized capillary tubes, which were sealed and immediately stored on ice, before centrifuging at 16 060g for 5 min later that day (median = 3.8 h after collection, s.d. = 2 h). Red blood cell fractions were stored in ethanol at 4°C for DNA analyses and parental assignment, while plasma fractions were frozen at –20°C for immunological assays. At the end of each fieldwork season, plasma samples were moved to –80°C.

(c) Immune indices

We assayed NABs, CA and Hp, which comprise part of the front-line immune defences. Respectively, these immune indices identify, eliminate and mitigate the threats posed and damage caused

by parasites and pathogens [42–44]. NABs are present in animals prior to any antigenic exposure and non-specifically identify a broad range of bacterial, viral and fungal antigens, providing broad-spectrum surveillance [55]. NABs bind to foreign antigenic components, opsonizing them for phagocytosis, and initiate the complement system via the classic pathway [56]. CA then aids the elimination of infection through a suite of activated proteins that lyse and break down the pathogen [44,57]. Where erythrocyte damage is sustained as a consequence of infection, Hp-like haem-binding scavengers (Hp or PIT54, an avian analogue) bind to reactive oxidative haem groups released to mitigate further damage done by infection [43,58]. These scavengers are tightly linked to the acute phase response and increase multiple-fold during infection and inflammation; however, constitutive levels can predict the strength of an immune response [59]. Assay methods are fully described by Roast *et al.* [60].

NABs and CA were quantified as agglutination and lysis, respectively, using the haemolysis-haemagglutination assay with minor modifications [60,61]. Inter-plate standards were scored for agglutination (mean = 10.1, $n = 247$) and lysis (mean = 3.55, $n = 265$) titres, respectively, resulting in a coefficient of variation (CV) = 0.13 and CV = 0.11. Hp was assayed using a commercial kit (Phase™ Range, TP801; Tri-Delta Development Ltd.) with a modified protocol [60]; inter-plate variation (CV = 0.24, $n = 25$ plates) was based on standards run in triplicate. From the initial samples, 82 fell above the 1.25 mg ml⁻¹ optical saturation threshold of the assay and were excluded from further analyses. Compared to other species (table 1 of [62]), NAB agglutination titres are relatively high in purple-crowned fairy-wrens (mean = 14.8, s.d. = 1.9), as are Hp concentrations (mean = 0.62 mg ml⁻¹, s.d. = 0.27), while CA lysis titres are at comparable levels (mean = 2.3, s.d. = 1.8).

(d) Survival

Survival was estimated as a binary variable (survived (1) or died (0)) based on whether a sampled individual survived until the next fieldwork season census (greater than 90 days post-capture). Our estimate of survival is close to the 'true' survival outcomes of individuals for several reasons. In the core population, individuals that disappear were declared dead only after all other group members were observed three times. Birds initially declared dead but then rediscovered within the core study population were very uncommon, with a 98% detection rate of individuals per fieldwork season in the core population. Furthermore, this species has limited dispersal abilities, dispersing only along waterways [63]. To find dispersers, 71% of all suitable habitat in a 40 km radius of the core population ([64]; electronic supplementary material, figure S2) was surveyed annually using an audio playback method (90% detection rate; [65]). These approaches allow us to distinguish dispersal from death with high accuracy.

(e) Reproductive success

Reproductive success was determined by matching free-flying juveniles to their putative parents using genetic data (for details see [52,65]). All juveniles that survived to independence were deemed recruits (greater than 90 days post-hatching; [52]) and the genetic parents of these recruits were considered to have successfully reproduced. Hatch dates were estimated for all recruits from direct nest observations or based on begging behaviour, morphological and plumage traits of known age of acquisition. To assess the cost of immunity on subsequent reproductive success for each individual sampled, all recruited offspring whose hatch dates fell during or after that fieldwork season were counted towards the individual's reproductive success (range 0–4 recruits). As only socially dominant individuals breed [50], all adults that were dominant at the time of sampling

that did not raise offspring to 90 days were assigned a 0 score for reproductive success. Subordinate individuals were also therefore excluded from reproductive success analyses, with the exception of seven individuals that were sampled as subordinates, but acquired dominant positions *and* then successfully bred before the next fieldwork season. Owing to low overall nesting success rate (12.7%; electronic supplementary material, figure S3), reproductive success data were highly zero-inflated and transformed to a binary variable of success (1) or failure (0).

(f) Dominance acquisition

In order to reproduce, acquisition of a dominant breeding position is crucial, and competition is high, with many subordinates dying before acquiring dominance (46%). Subordinates can form stable queues within groups to inherit a dominant position [66], and in males at least, ornaments are associated with the probability to acquire a breeding position [67]. Dominance acquisition was recorded as a binary variable of success (1) or failure (0) in acquiring a dominant breeding position during or after the fieldwork season in which the subordinate individual was immune sampled, prior to the subsequent fieldwork season.

(g) Statistical analysis

All analyses were carried out in the R statistical environment (v. 3.6.0; [68]). To address the key questions of this study, immune indices were treated as explanatory variables to all fitness-related response variables—survival, reproductive success and dominance acquisition. This model arrangement, in addition to the temporal lag in the quantification of response variables (outcome after immune measurements were taken) imply a cost of immune function, though causality cannot be inferred from this observational study. Because constitutive immune function may be costly, it could be expected that some intermediate, rather than maximal, level of constitutive immunity is optimal for fitness; we therefore test by default the quadratic relationships between each immune index and all fitness-related traits. To use immune indices as explanatory variables, raw values were first corrected for sources of measurement error with linear mixed-models (LMMs) using *lme4* [69]. In separate LMMs (Hp and NABs were normally distributed, CA was natural log transformed to comply with assumptions with normality), the following potential sources of measurement error were included as fixed effects in each model: time bled—the time of day relative to sunrise [70]; time wait—the delay between capture and sampling representing handling stress [70,71]; fieldwork season—which accounts for both inter-annual and time of year (May/November) differences, in addition to any storage differences between fieldwork season sample batches; a random effect of plate identity (ID) controlled for assay inter-plate variation in each model. Residual values from these models were considered 'corrected' immune index values, and these values were used as explanatory variables in all further analyses. Corrected immune index values showed only weak pairwise correlations (Hp-NABs: $r = 0.27$, $p < 0.001$, $n = 454$; Hp-CA: $r = -0.12$, $p = 0.012$, $n = 453$; NABs-CA: $r = -0.12$, $p = 0.009$, $n = 509$). In addition, to avoid conflation of the reproductive success and dominance acquisition responses with survival, only birds that survived to the next fieldwork season were retained in analyses involving reproductive success and dominance acquisition.

Generalized linear mixed-effects models (GLMMs) were used to assess the relationship between immune indices and the fitness-related variables using the *lme4* package [69]. As all response variables were binary, logistic regression mixed models with binomial error structures were applied, with individual ID as a random effect to account for non-independence of repeated measures. Because not all individuals had the complete panel of

indices available, for each fitness-related response, separate GLMM models were constructed using all available samples for each immune index. Age at capture and sex were also included in all models as covariates as these may influence short-term fitness outcomes. Additionally, the time of year birds were sampled (May or November fieldwork season) was included in models with reproductive success as a response, since this was consistently higher after November sampling as birds entered the wet season breeding peak. To each of these models, a quadratic term of each corrected immune index was also included to test for nonlinear effects, but removed if not significant ($p > 0.05$) using *lmerTest* [72]. In each survival GLMM, models experienced convergence and fitting issues, which were resolved by removing the individual ID random effect. In such cases, the equivalent generalized linear model (GLM) without the individual ID random effect was fitted to obtain estimates, but as GLMs do not fully control for non-independence of data points, results of these models need to be interpreted with a degree of caution.

Two physiological variables that can covary with immune function and also be consequential for fitness outcomes are body condition and chronic stress [73–75]. Within our dataset, a subset of samples had data available for metrics of body condition and/or chronic stress. To control for any covariation between immune function and fitness that might be condition- or stress-dependent, body condition and chronic stress variables were separately added to each logistic regression model assessing the influence of immune indices on fitness-related outcomes, though in each case with a reduced sample size (electronic supplementary material, table S1, body condition; electronic supplementary material table S2, chronic stress). Body condition was calculated as the residuals of a linear regression model of body mass at capture, corrected for individual tarsus length and time of day (a body condition metric qualitatively similar to ‘scaled mass index’ for purple-crowned fairy-wrens; electronic supplementary material, table S2 of [52]). Chronic stress was quantified using heterophil-lymphocyte (HL) ratios [76] that were scored from blood smears collected at the same time as immune sampling (for full method description see [60]). Similar to immune indices, raw HL ratios were first corrected for time bled, time wait, fieldwork season, and as a random effect, scorer ID, before the inclusion of model residuals as an explanatory variable. Controlling for either body condition or chronic stress where data were available did not alter the outcomes of the main analyses (electronic supplementary material, tables S1 and S2), nor did body condition or HL ratio directly influence fitness outcomes in these models; these analyses are therefore not reported further.

Finally, for each fitness-related response, one additional model containing all corrected immune indices, chronic stress and body condition as explanatory variables combined was constructed to validate the results obtained in the models with the immune indices separately; these three combined models had substantially reduced sample sizes compared to any of the independent models (table 1 for details). The outcomes of these combined models were congruent with models presented here, and details are reported in the electronic supplementary material only; figures S4 and S5 and tables S3–S5.

3. Results

(a) Survival

CA was the only index to significantly predict survival (table 2 and figure 1c). Interestingly, it showed a quadratic effect, with intermediate values of CA having the lowest predicted probability of survival, while individuals that had either higher or lower values were more likely to survive to the next fieldwork season (table 2; $\beta = 0.525$, 95% confidence

Table 1. Matrix of sample sizes (n) for model configurations. (From a total of 716 captures of 351 adult individuals (greater than 90 days), sample sizes for each immune index differ because not all immune indices could be quantified from each plasma sample. Reproductive success analyses include only dominant individuals, and dominance acquisition analyses include only subordinate individuals. The ‘combined’ column counts only the subset of samples for which all immune, chronic stress and body condition variables were available.)

		explanatory			
		Hp	NAbs	CA	combined
response	survival	648	520	519	337
	reproductive	305	248	250	156
	success				
	dominance	290	235	231	158
	acquisition				

interval (CI) = 0.098, 1.027). Furthermore, individuals with higher NABs were less likely to survive, and although the effect was fairly large, with 24% decrease in survival per s.d. increase in NABs, this result was not significant (table 2; $\beta = -0.276$, 95% CI = -0.621 , 0.063 ; figure 1b). Neither age or sex had any effect on survival (electronic supplementary material, table S5).

(b) Reproductive success

For reproductive success, NABs appear to be the most important explanatory variable (table 2; $\beta = 0.563$, 95% CI = 0.219 , 0.962), with a 76% increase in the probability to successfully produce at least one recruit, per s.d. increase in NABs (table 2 and figure 1e). There was also a substantial, but not significant, increase in the probability of reproductive success with increased Hp (table 2; $\beta = 0.254$, 95% CI = -0.040 , 0.574 ; figure 1d), while CA had only negligible effects. The probability of reproductive success was not affected by age, but was slightly higher among sampled females relative to males in NABs and CA models (electronic supplementary material, table S5). Time of year showed that individuals sampled in November had a higher probability of reproductive success in the NABs model (electronic supplementary material, table S5), as expected owing to the wet season breeding peak that follows the November fieldwork season (electronic supplementary material, figure S1).

(c) Dominance acquisition

The probability of successfully acquiring a dominant breeding position was not significantly related to any immune index. Although Hp concentrations had the largest observed effect of any immune index on the probability of acquiring a breeding position in the following months, CIs overlapped zero (table 2; $\beta = 0.267$, 95% CI = -0.046 , 0.580 ; figure 1g). Dominance acquisition was not affected by age, but in each model, it was more probable that females acquired dominant breeding positions compared to males (electronic supplementary material, table S5). The sex effects on fitness outcomes (both dominance acquisition and reproductive success) are probably a consequence of female-dominated dispersal in this species, where females seek out breeding vacancies in

Figure 1. The effect of individual variation in immune indices on subsequent fitness-related outcomes in purple-crowned fairy-wrens. Fitted lines show the predicted probability of survival (*a–c*), successful reproduction of at least one recruited offspring (*d–f*), and acquisition of a dominant breeding position (*g–i*), in response to (from top to bottom) haptoglobin (Hp), natural antibodies (NAbs) and complement activity (CA). Ribbons show confidence intervals based upon error of fixed effects only, while all continuous variables are held at median values. Lines are shown for males in May only, as reference categories for ‘sex’ (all responses) and ‘time of year’ (reproductive success response only). Lines and ribbons are derived from models fitted independently with a single index and maximum sample. Data ticks bordering each plotting space show raw data values at binary 1 and 0 outcomes; y-axes are cropped to improve visualization of the fitted lines. Plots labelled with ‘*’ denote significant effects of indices in final models.

Table 2. The effects of immune indices on fitness-related traits. (The β -estimates and 95% confidence intervals (CI) are derived from independent models for each immune index and are presented on the logit link function scale. Odds ratios (OR) and the percentage change in the probability of the respective response ($\Delta\% p(\text{response})$) are per s.d. in the corrected immune indices. Italic typeface shows variables that were significant, determined by 95% CI that do not contain zero and $p < 0.05$ for z-tests (electronic supplementary material, table S5).)

response	explanatory	β	(95% CI)	OR	$\Delta\% p(\text{response})$
survival	haptoglobin	−0.150	(−0.412, 0.120)	0.86	−14%
	natural antibodies	−0.276	(−0.621, 0.063)	0.76	−24%
	complement activity	0.252	(−0.136, 0.673)	1.29	29%
	<i>(complement activity)²</i>	<i>0.525</i>	<i>(0.098, 1.027)</i>	<i>1.69</i>	<i>69%</i>
reproductive success	haptoglobin	0.254	(−0.040, 0.574)	1.29	29%
	<i>natural antibodies</i>	<i>0.563</i>	<i>(0.219, 0.962)</i>	<i>1.76</i>	<i>76%</i>
	complement activity	−0.013	(−0.316, 0.298)	0.99	−1%
dominance acquisition	haptoglobin	0.267	(−0.046, 0.580)	1.31	31%
	natural antibodies	−0.014	(−0.382, 0.354)	0.99	−1%
	complement activity	−0.203	(−0.589, 0.182)	0.82	−18%

other territories, whereas males will often 'queue' in their natal territories to inherit a breeding position.

4. Discussion

(a) High constitutive immune function does not predict survival

We predicted that as a consequence of parasite pressure, higher immune function would be directly related to a higher probability of survival. In relation to our three constitutive immune indices, this was not the case, despite their important roles for front-line defences. Hp and NAbS were unrelated to survival probability, while we found a significant quadratic effect of CA. We tested for quadratic effects in these models expecting a possible optimal level of immune function; however, instead of a peak for optimal immunity, we unexpectedly observed a trough, showing that intermediate values had the lowest probability of survival. This could suggest a bimodal (high and low) level of optimal CA, potentially determined by temporal, spatial or parasitological environmental variability [77–79]. For example, there may be fluctuations in microbial or vector abundance with seasonal rainfall, because territories vary in presence and size of persistent waterholes during the dry season [80–83], or there may be localized outbreaks of specific parasites in the parasite-rich riparian habitat [84]. Regardless, the lack of a simple correlation between our immune assays and survival, in combination with the very low within-individual repeatability of immune measurements in this species [60], suggests that immune responses are highly plastic and strongly environmentally determined.

(b) No trade-off between immune function and reproduction

We hypothesized that investment in immune function would result in a trade-off with reproduction as both traits compete for a limited pool of resources (though obligatory trade-offs can also result from coupled immune and reproductive pathways; [85]). Therefore, we expected immune function to be inversely related to subsequent reproductive success, yet this was unsupported for all of our immune indices. Higher levels of NAbS strongly predicted greater likelihood of reproductive success, and the same trend was evident for Hp, although non-significant. These results indicate that the expression of both reproduction and immunity could positively correlate with a third unmeasured variable, an individual or environmental 'quality', obscuring any resource reallocation trade-off. High-quality individuals might inherently be able to invest more in both immunity and reproduction [86,87], be more efficient foragers [88], or holders of superior territories with better resource access [89]. Any trade-off that exists between immune function and reproduction may thus become overwhelmed by the large variation in the acquisition of resources among individuals in the population, in agreement with life-history theory models that predict an absence of evidence for trade-offs at the population level [90]. A genetic basis for such differences in individual quality might exist in purple-crowned fairy-wrens, which are particularly vulnerable to inbreeding because severe habitat fragmentation and limited dispersal ability have reduced genetic diversity in isolated subpopulations

[91], while incestuous dominant pairings are highly costly to reproductive success [51,65]. Consequently, as inbreeding has been shown to cause immunosuppression [92], this could result in positive correlations between individual genetic diversity, reproductive success and immune function.

We investigated whether covarying expression of immune function and reproduction was explained by body condition, a proxy for energetic resources, or HL ratio, an index of chronic stress, in the models of our fitness responses (electronic supplementary material, table S1). However, we found no positive relationship between body condition or chronic stress and reproductive success, i.e. no underlying condition or stress dependence. Together with no evidence of a trade-off between immune function and reproductive success, this suggests there is unlikely to be a strict constraint (energetic or stress-related—e.g. anti-oxidant) that prohibits both maintenance of constitutive immune function and reproductive activity. As purple-crowned fairy-wrens reproduce only when conditions are most favourable and there is an abundance of invertebrate prey [52], energetic constraints might still be quite relaxed even when physiological demands of breeding are high, and constitutive immune function can be maintained throughout the annual cycle [60], irrespective of individual stress or reproductive state. Alternatively or additionally, the demands of maintaining constitutive immune functions may be too low to invoke strong trade-offs. The immune-reproductive trade-offs consistently found in birds stem from negative effects of immune challenges that generate an acute immune response, rather than constitutive immune function, and trade-offs might only become apparent when the individual is challenged [22,93]. However, despite the consistent evidence for effects of immune system activation on reproduction, the proximate currency mediating those costly effects remains mysterious, and it seems unlikely this is energy or nutrient based (meta-analysis: [22]). Possibly the correlations between constitutive immunity and reproduction differ from the effect of induced immune responses on reproduction not because of differences in costs, but as a result of adaptive reallocation away from investing in reproduction when the organism is challenged by an infection or risk of infection of subsequent offspring.

(c) Immune function does not predict dominance acquisition

Our hypothesis that the probability of dominance acquisition would be a function of individual quality and, therefore higher for individuals with higher immune levels, was not supported. No immune index significantly related to the probability of dominance acquisition (table 2). In male purple-crowned fairy-wrens, the probability of acquiring a dominant breeding position is related to male ornamentation [67]. Although the adaptive role of female ornamentation is not fully understood [94], both sexes exhibit distinct seasonal plumage colour, which is either structural (males) or melanin-based (males and females) [95]. These types of coloration can honestly signal components of individual quality [96–98], possibly including immunocompetence [99]. Although we do not fully understand how ornamentation relates to individual quality in purple-crowned fairy-wrens, our results on immune function suggest that the mechanisms which convey individual quality through plumage ornamentation

may not be the same as those which relate to constitutive immune function.

5. Conclusion

Unrelenting parasitic and pathogenic selective pressures ensure that constitutive immune defences remain integral to both vertebrate and invertebrate immune systems and ultimately fitness. We made point measurements of potentially dynamic constitutive immune indices and tested whether these were related to individual fitness-related outcomes in the following weeks/months. Surprisingly, we found that survival was not linearly related to high levels of constitutive immune indices. In addition, positive relationships between reproductive success and immune indices—significantly NAbS—suggest that between-individual heterogeneity in some unmeasured aspect of quality or condition is more important than resource allocation trade-offs within individuals. Without evidence of an immune-reproductive trade-off, our results suggest the fitness costs of maintaining these constitutive humoral immune components may not be as substantial as the costs of induced or cellular immune responses that invoke trade-offs in other systems. Moreover, if costs are commonly associated with particular immune components not found in invertebrate or more basal immune systems, then trade-offs might not be invoked at all in these animals. Immune component-specific costs are not a new concept [23], yet quantifying the physiological costs of constitutive immune components, that are not amenable to experimental

manipulation, has been a difficult task for ecoimmunologists and remains a challenge. Once we develop a more detailed understanding of precise energetic, nutritional, metabolic and oxidative patterns of covariation of specific immune components, we will be able to hypothesize more precisely about how individual variation in immune function relates to fitness outcomes and evolutionary host-parasite dynamics. Alternatively, perhaps immune plasticity of individuals would better predict fitness outcomes in highly changeable environments.

Ethics. All relevant permissions for this research were acquired from the Australian Bird and Bat Banding Scheme (licence 2230), Western Australia Department of Parks and Wildlife and the Monash University School of Biological Sciences Animal Ethics Committee.

Data accessibility. Data and analysis code can be found on the Dryad Digital Repository: <https://dx.doi.org/10.5061/dryad.2jm63xsxk> [100].

Authors' contributions. M.J.R. and A.P. conceived the study design. M.J.R., N.H.A., M.F. and N.T. collected field data. M.J.R., M.D.H. and A.P. conducted the data analyses. All authors contributed to the interpretation and discussion of the results, in addition to the preparation of the manuscript.

Competing interests. We declare we have no competing interests.

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