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RESEARCH ARTICLE

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Differential recovery ability from infections by two blood parasite genera in males of a Mediterranean lacertid lizard after an experimental translocation

R. Megía-Palma[1,2,3] | L. Redondo[4,5] | S. Blázquez-Castro[1] | R. Barrientos[4]

[1]Universidad de Alcalá (UAH), Department of Biomedicine and Biotechnology, Parasitology, Alcalá de Henares, Spain

[2]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, Vairão, Portugal

[3]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, Vairão, Portugal

[4]Road Ecology Lab, Departamento de Biodiversidad, Ecología y Evolución, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, Madrid, Spain

[5]Biodiversity Node S.L., Madrid, Spain

Correspondence

R. Megía-Palma, Universidad de Alcalá (UAH), Department of Biomedicine and Biotechnology, Parasitology, E-28805, Alcalá de Henares, Spain.
Email: rodrigo.megia@uah.es

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Abstract

Different blood parasites can co-infect natural populations of lizards. However, our knowledge of the host's ability to recover from them (i.e., significantly reduce parasitemia levels) is scarce. This has interest from an ecological immunology perspective. Herein, we investigate the host recovery ability in males of the lizard *Psammodromus algirus* infected by parasite genera *Schellackia* and *Karyolysus*. The role of lizard hosts is dissimilar in the life cycle of these two parasites, and thus different immune control of the infections is expected by the vertebrate host. As *Schellackia* performs both sexual and asexual reproduction cycles in lizards, we expect a better immune control by its vertebrate hosts. On the contrary, *Karyolysus* performs sexual reproductive cycles in vectors, hence we expect lower immune control by the lizards. We carried out a reciprocal translocation experiment during the lizards' mating season to evaluate both parasitemia and leukocyte profiles in male lizards, being one of the sampling plots close to a road with moderate traffic. These circumstances provide a combination of extrinsic (environmental stress) and intrinsic factors (reproductive vs. immune trade-offs) that may influence host's recovery ability. We recaptured 33% of the lizards, with a similar proportion in control and translocated groups. *Karyolysus* infected 92.3% and *Schellackia* 38.5% of these lizards. Hosts demonstrated ability to significantly reduce parasitemia of *Schellackia* but not of *Karyolysus*. This suggests, in line with our predictions, a differential immune relationship of lizards with these parasites, at time that supports that parasites with different phylogenetic origins should be analyzed separately in investigations of their effects on hosts. Furthermore, lizards close to the road underwent a stronger upregulation of lymphocytes and monocytes when translocated far from the road, suggesting a putative greater exposure to pathogens in the latter area.

KEYWORDS

ecological immunology, *Karyolysus*, *Psammodromus algirus*, *Schellackia*, white blood cells

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1 | INTRODUCTION

Despite parasitism being defined as an ecological relationship with negative net effect on the host's fitness, different parasites may follow different strategies to exploit their hosts. This can result in specific co-adaptation histories that may lead to immune avoidance strategies in some parasites (Lagrue et al., 2016; Schmid-Hempel, 2008). We may thus expect that the clearance efficacy of antiparasitic strategies vary for different parasites. Although this is of interest from both ecological immunology and conservation (e.g., disease control) perspectives, our knowledge of the ability of hosts to recover from parasitic infections is scarce in most host–parasite systems. This is the case of lizard hosts in the family Lacertidae and the various blood parasites that naturally co-infect them. To advance scientific knowledge in this regard, longitudinal experiments (i.e., those in which parasite prevalence is measured at different times under controlled conditions) are essential. With the advent of molecular methods in the last decade, we have been able to match taxonomic identity of protozoan blood parasites of lizards with morphology (phylum Apicomplexa) (Megía-Palma et al., 2013). For example, a recent survey of over 700 lizards in the family Lacertidae revealed that *Karyolysus* (Apicomplexa: Adeleorina) is the most common parasite genus in Iberian lacertids (Megía-Palma et al., 2023). Blood cells infected by this parasite have nuclei that usually present a distorted morphology (Haklová-Kočíková et al., 2014; Reichenow, 1919). *Schellackia* (Apicomplexa: Eimeriorina) is the second most frequent parasite genus (Megía-Palma, Martínez, Cuervo, et al., 2018) and its blood stages distinctly present one refractile body (Megía-Palma et al., 2014; but also see Megía-Palma et al., 2017). These two genera of protozoans produce chronic infections that last for years in the blood of lizards (Sorci, 1995). However, *Karyolysus* and *Schellackia* may differ in their host exploitation strategies. *Karyolysus* undergoes asexual reproduction in several organs and tissues of lizards including the liver, lung, heart, ovaries, and spleen (Svahn, 1975), whereas sexual reproduction occurs in an hematophagous mite of the genus *Ophionyssus* (=*Sauronyssus*) (Acari: Macronyssidae) (Megía-Palma et al., 2023). This arthropod acts as vector (Haklová-Kočíková et al., 2014). A positive covariation of *Karyolysus* parasitemia and male nuptial coloration has been reported for the lacertid species *Podarcis muralis*, *Gallotia galloti*, and *Psammodromus algirus* (Martín et al., 2008; Megía-Palma et al., 2016; Megía-Palma, Merino, et al., 2022). This can be interpreted as a cost associated with male reproductive effort (Badiane et al., 2022; Megía-Palma et al., 2021). In contrast, *Schellackia* primarily infects the intestine walls of lizards (Bristovetzky & Paperna, 1990). However, sporozoites of *Schellackia* can migrate through mesenteric vessels and invade liver cells and peripheral blood, where their effects are poorly known (Bristovetzky & Paperna, 1990). The close phylogenetic relationship of *Schellackia* with intestinal coccidians of the order Eimeriorina (Megía-Palma et al., 2013, 2014) presupposes that infection by blood eimeriorines might have negative impacts similar to those associated with intestinal ones. The latter includes increased susceptibility to infections by other pathogens (in mammals; Bertó-Moran et al., 2013), increased cellular oxidative stress (in birds; Sepp et al., 2012), histopathological clinical signs and increased susceptibility to bacteria (in fish; Solangi & Overstreet, 1980; Steinhagen et al., 1997), reduced packed cell volume (in amphibians; Mitchell, 2007), and disrupted thermoregulatory behavior or duller sexual coloration (in other reptiles; Megía-Palma, Paranjpe, Reguera, et al., 2018; Megía-Palma, Paranjpe, et al., 2020). In lizards, negative longitudinal covariation between *Schellackia* parasitemia and nuptial coloration has been described in *P. algirus*, particularly in interaction with between-year changes in mite load (Megía-Palma, Merino, et al., 2022). Similarly, a greater recovery ability was demonstrated in males of *Lacerta schreiberi* infected by *Schellackia* only after experimental clearing of their tick load (Megía-Palma, Martínez, Merino, 2018). These two results suggest that maintaining a low parasitemia of *Schellackia* may be an energetically demanding process for hosts that can compete with production of male nuptial coloration even in mid-sized hosts, such as *L. schreiberi*, where males are relatively robust and average 25 g.

Research highlights

- Host recovery ability may differ for different parasites.
- We studied two genera of blood parasites in male lizards.
- Host recovery was demonstrated only against one of the parasites.
- A differential immune relationship with these parasites is suggested.

Schellackia performs both asexual and sexual reproductive cycles in lizard definitive hosts (Megía-Palma et al., 2013) and may be transmitted by hematophagous mites. However, these arthropods may only act as mechanical transmitters for *Schellackia*, because the parasite stages become dormant (i.e., hypnozoites) within the mite's body, only becoming infective when lizards swallow and digest the mite (Telford, 2009). Therefore, *Karyolysus* can reproduce both in invertebrate and vertebrate hosts, whereas *Schellackia* can only do it in lizard hosts. Interestingly, recent research using similar methodologies have found differential cophylogenetic signals between lacertid host species and both *Schellackia* and *Karyolysus* (Megía-Palma, Martínez, Cuervo, et al., 2018; Megía-Palma et al., 2023). These studies suggest that the lacertid phylogenetic radiation in the western Mediterranean has a significant influence on *Schellackia* diversification process, whereas there was no statistical support for a similar influence on *Karyolysus* radiation (Megía-Palma, Martínez, Cuervo, et al., 2018; Megía-Palma et al., 2023).

We hypothesize that the observed differences in cophylogenetic histories and life cycles of the two parasites can result in differential immune relationships and a dissimilar recovery ability from infections by *Schellackia* and *Karyolysus* in lizards. We predict a better host recovery ability (more

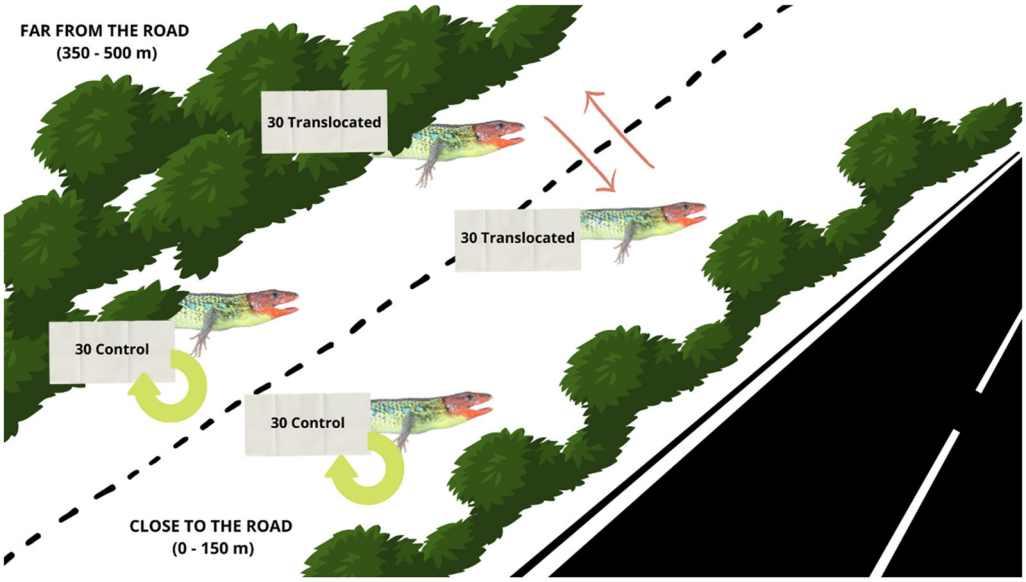

FIGURE 1 Experimental design where 60 males were randomly assigned into control or translocation treatments per sampling area (terrain bands of 150 m of width). Control lizards were released and recaptured in the same area, whereas translocated lizards were released and recaptured in the opposite area. The dashed line represents a 200 m-wide area that was left unsampled and served as separation between sampling areas.

parasitemia reduction) from the infection by *Schellackia*, because this parasite performs its entire reproductive cycle in the lizards. For this reason, we hypothesize a narrower host–parasite co-adaptation that can enable a better control of *Schellackia* replication by the lizards. To test this hypothesis, we investigated parasitemia (number of infected blood cells/total of cells estimated) and its longitudinal variation, which is a proxy to host recovery ability in lizards (e.g., Foronda et al., 2007). We carried out a full-cross capture–translocation–recapture experiment performed in a population of the lacertid lizard *P. algirus* during its mating season (Barrientos & Megía-Palma, 2021). One of the sampling zones is close to a road and the quality of the vegetation is worse than in the second sampling zone, which is farther from the road (Megía-Palma, Merino, et al., 2022). This provides the opportunity to also investigate potentially additive effects associated with reproductive effort and anthropogenic environmental stress on the recovery ability of male lizards. This is why we will also analyze cellular components of the host immune system, because leukocyte profiles and total white cell (WC) counts are considered measures of immune status associated with stress (Davis et al., 2008; Han et al., 2020). We predicted that leukocyte profiles of the lizards will show further costs to those already described on body condition and associated with translocation (see Barrientos & Megía-Palma, 2021). Finally, we also expect trade-offs between the recovery ability from parasitic infections and color patch production (a proxy to reproductive effort in males; Badiane et al., 2022), where those males that increase coloration will perform worse at reducing blood parasites.

2 | MATERIAL AND METHODS

2.1 | Sampling and morphometric variables

We investigated *P. algirus*, a species with generalist habits with a distribution across the Iberian Peninsula and North of Africa (Llanos-Garrido et al., 2021). The investigation was performed in an evergreen forest where vegetation is dominated by holm-oak trees (*Quercus ilex*) and gum rockrose bushes (*Cistus ladanifer*). It falls within the Mesomediterranean bioclimatic region in the center of the Iberian Peninsula (40.5063°N, 3.7662°W; ~680 m above sea level) (Rivas-Martínez et al., 2017). We used a lasso to capture male lizards between April 24 and July 6, 2018. Males were identified due to their enlarged femoral pores, longer tails and more volumetric heads, and orange coloration. We measured the snout-vent length (*aka* "body length") with a ruler to the nearest millimeter. We weighed (i.e., calculated "body mass") the lizards with a digital scale to the closest 0.01 g. We calculated a "body condition" index as the residuals of the regression between log10-transformed values of both body mass and body length. Positive residuals refer to lizards carrying more weight per unit body length (heavier) versus lizards with lower mass per unit body length (lighter).

2.2 | Experimental design

We sampled lizards in two sampling zones separated by more than 200 m one from another. This distance exceeds the home range of the species investigated (Díaz, 1993) and the homing distance of medium-sized lacertids (Strijbosch et al., 1983). These two sampling

zones differed in their proximity to a road (9050 vehicles/day). The first zone ranged between zero and 150 m from a road, whereas the second one ranged between 350 and 500 m from it (Barrientos & Megía-Palma, 2021). In each of these two sampling zones, the individuals were randomly assigned to one of two treatments: (i) "control" lizards, which were released in the XY location where they were captured, either close or far from the road; and (ii) "translocated," which included lizards released in the opposite zone. Thus, four groups of lizards were created: control close to the road, control far from the road, translocated close to the road, and translocated far from the road (Figure 1).

We captured lizards during 18 sampling days, between April 24 and June 27. Lizards were searched randomly by continuously resampling the study areas and were recaptured at least fourteen days after their first capture (range = 14–43 days). We extended the recapture effort for five more days until July 6. Resampling effort was homogeneous between the two sampling zones (no significant differences in resampling dates between zones: $F_{1,37} = 0.38$, $p = 0.54$; no significant differences in number of elapsed days between capture and recapture between zones: $F_{1,37} = 0.56$; $p = 0.46$). Three strategies were followed in the field to recognize recaptured lizards: (i) individuals were marked on the back with a xylene-free white paint at capture; (ii) they were assigned an individual code by toe-clipping (Barrientos & Megía-Palma, 2021; Perry et al., 2011); (iii) the individuals were photographed, which allowed the verification of the identity of the individuals in the case they had eventually lost extra phalanxes by comparison of the scales of the lateral of the head in the pictures made at capture and recapture of the individual suspicious to be the same based on the ID code assigned by toe-clipping at first capture (Megía-Palma, Merino, et al., 2022).

2.3 | Distance travelled by lizards

We referenced the point where we captured the lizards using a GPS device (eTrex, Garmin). We calculated "travelled distance" as the Euclidean distance between capture and recapture points (Barrientos & Megía-Palma, 2021).

2.4 | Leukocyte counts

We collected blood samples from the tail using sterile needles (Megía-Palma, Merino, et al., 2022). We smeared the blood samples (~5 μL) on microscope slides that were air-dried, fixed with methanol, and stained with Diff-Quick (Nardini et al., 2013). We used a light optic microscope (BA410, Motic) to differentially count white blood cells in 50 microscope fields at ×1000 magnification and with a homogeneous distribution of erythrocytes. We discriminated heterophils, eosinophils, basophils, lymphocytes, monocytes, and azurophils based on Puerta et al. (1996). We calculated the proportion of every WC type and total WC counts in relation to estimated total number of erythrocytes in 50 microscope fields. For this reason, we counted the number of erythrocytes in two randomly selected microscope fields in every blood smear (mean ± SD = 131.78 ± 10.23 erythrocytes/field). We performed a factor analysis with the estimates of leukocyte to (i) reduce model over parameterization and (ii) avoid model autocorrelation (Veiga et al., 1998). After a varimax rotation of the axes, the principal components analysis produced two principal factors with eigenvalue > 1 that together explained 61% of the initial variance in WCs. PC1_WC explained 39% of the variance and positively correlated with monocytes, lymphocytes, and total WC. PC2_WC explained 22% of the variance and was strongly correlated with heterophils, and had a more moderate positive correlation with basophils, eosinophils, and azurophils (Supporting Information: Table S1).

2.5 | Quantification of parasites

We quantified ectoparasites (mites) at capture and recapture using a magnifying glass (×10) in the field (Barrientos & Megía-Palma, 2021). We used morphology at ×1000 magnification in the same microscope than leukocyte profiles to discriminate blood parasites as follows: *Schellackia* has relatively smaller infective stages in the blood (sporozoites) and show one bluish refractile body. *Karyolysus* was distinguished by its banana-like shape, the presence of a parasitophorous vacuole, digestive vacuoles in trophozoites, and its distorting effects on both host cell membrane and nucleus. Parasitemia of the two parasites was calculated as the proportion of infected erythrocytes in an estimated total number of erythrocytes in 100 microscope fields (Megía-Palma et al., 2023).

2.6 | Quantification of nuptial coloration

Males of *P. algirus* in this population have UV-based lateral eyespots, a yellow throat, and orange coloration on the mouth commissure that, in some males, can expand and cover a large proportion of the head (Megía-Palma, Merino, et al., 2022). We calculated a spectral color distance index (SCD) (i.e., Endler, 1990) of every lizard as the Euclidean distance between the spectral variables of these three color patches as follows:

$$\mathrm{SCD} = \sqrt{(\Delta\ \mathrm{hue})^2 + (\Delta\ \mathrm{chroma})^2 + (\Delta\ \mathrm{luminance})^2}.$$

We used a spectrophotometer with ultraviolet light spectrum sensitivity (Jaz DPU® Module, Ocean Optics Inc.) that was connected to a pulsed xenon lamp source. We quantified the spectral reflectance of the orange patch on the right commissure of the mouth, the yellow patch on the throat, and the blue patch on the first and largest eyespot in the right flank of the lizards. For the spectral readings, we used a glass-fiber probe that was inserted in a black holder that reduced noise from environmental light (for further methodological details see Supporting Information: Materials).

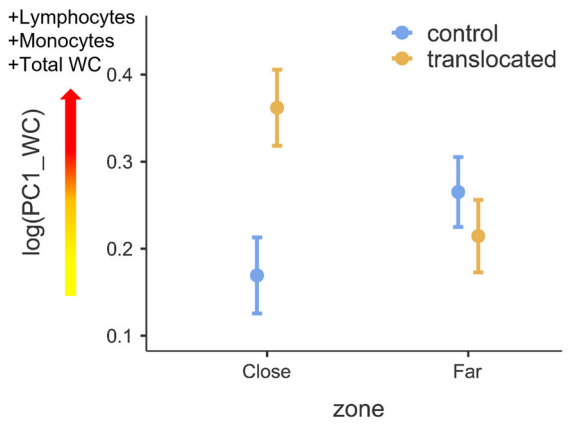

FIGURE 2 Interaction plot based on model residuals showing the differential effects on leukocyte profiles of *P. algirus* upon the translocation treatment depicted in Figure 1. A greater effect was found for the lizards captured closer to the road: translocated lizards suffered a strong increase in total WC, lymphocytes, and monocytes (see Supporting Information: Table S3). Labels in the *X* axis depict the origin of the lizards.

2.7 | Statistical analyses

All analyses were performed in R 4.0.4 (R Core Team, 2021). To analyze effects of the experiment on WC components (PC1_WC and PC2_WC) and parasitemias of *Schellackia* and *Karyolysus*, we fitted four generalized mixed models (GLMM; Cnaan et al., 1997), one for each leukocyte principal factor and one for each parasite genus. They had Gamma residual distribution and inverse linking function after adding a random value of two units to all response scores and later $\log_{10}$ transforming these new values. This was done to avoid negative values that are not allowed in Gamma distributions. In the four models, we set as fixed predictors the interaction between treatment and sampling zone, capture event (capture or recapture), date of capture, body condition, number of mites, number of blood parasites of the other genus than the one tested in the model, the distance travelled by the lizards between capture and recapture, number of days elapsed between capture and recapture, and SCD. Leukocyte principal factors were only added to the models of parasites. The interaction between *Schellackia* and *Karyolysus* was included in the models of PC WCs to control possible effects of co-infection. We *z*-standardized all these predictors to improve computational stability and make coefficient scores comparable (Megía-Palma, Arregui, et al., 2020). We set as random terms in the GLMM the individual, the individual nested within the sampling plot, and the individual nested within the treatment (Megía-Palma, Merino, et al., 2022). We checked the residual distribution and the autocorrelation of the models based on variance inflation factor (which were <2 for all predictors in the four models) (Lüdecke et al., 2021).

Given the relatively large number of predictors, we applied a model selection methodology corrected for small sample sizes (corrected Akaike's Information Criterion [AICc]; Akpa & Unuabonah, 2011) and model averaging. This method reduces over parameterization, because it removes those predictors without effect (Hegyi & Garamszegi, 2011; Symonds & Moussalli, 2011). It was implemented with the R package MuMIn (Barton, 2018), and we calculated an averaged final model that considered all the models with a difference of AICc ≤ 2 (Burnham & Anderson, 2004). We performed a Bonferroni posthoc test to check significant differences of the interaction effects between translocation and zone in the model of PC1_WC (see Section 3).

3 | RESULTS

We initially captured 120 adult males, 60 per sampling zone. In addition, we were able to recapture 40 of them. However, we removed one lizard from the analyses, because one of the blood smears was spoiled during the staining process. Therefore, final sample size was 39 recaptured lizards (11 Control far + 10 Translocated far; and 9 Control close + 9 Translocated close).

3.1 | Leukocytes

Leukocyte counts showed a clear predominance of lymphocytes (mean ± SE = 66.5 ± 2.6%), followed by azurophils 15.2 ± 1.8%, heterophils 10.8 ± 1.9%, basophils 5.0 ± 0.6%, monocytes 2.4 ± 0.4%, and eosinophils 0.1 ± 0.1%. Model averaging produced three models with AICc ≤ 2 for PC1_WC that was explained by date ($\beta = -0.13 \pm 0.06$), elapsed days between capture and recapture ($\beta = +0.02 \pm 0.01$), and the interaction between treatment and zone (Supporting Information: Table S2 and Figure 2). Thus, total WC, lymphocytes, and monocytes decreased with capture date (i.e., seasonal decrease) but tended to increase in all the lizards between capture and recapture. Interestingly, according to the Bonferroni posthoc test (Supporting Information: Table S3), the strongest leukocyte upregulation occurred in those males from the zone close to the road. Therefore, total WC, lymphocytes, and monocytes increased the most in the males that were translocated to the zone farther from the road, as compared to control lizards from that same zone (Figure 2). Model averaging produced two models with AICc ≤ 2 for PC2_WC and none of the estimated effects were considered important (Supporting Information: Table S4).

3.2 | Parasites

Schellackia infected 31.7% of the initial sample of 120 lizards (0–157 blood parasites) and *Karyolysus* infected 80.0% of them (intensity range = 0–133 blood parasites) ($\chi^2 = 58.32$, $p < 0.001$). Similarly, in the 39 recaptured lizards, the prevalence of *Schellackia* (38.5%) was also

significantly lower at capture than that of *Karyolysus* (92.3%) ($\chi^2 = 24.98$, $p < 0.001$). Both values of blood parasite prevalence remained similar between capture and recapture (*Schellackia*$_{final}$ = 33.3%; $\chi^2 = 0.22$, $p = 0.64$; *Karyolysus*$_{final}$ = 87.2% – $\chi^2 = 0.56$, $p = 0.45$). Mites infested 100% of the recaptured lizards both at capture (16.2 ± 1.7 mites) and recapture (mean ± SE = 25.9 ± 2.6 mites).

Model selection produced eight models with AICc ≤ 2 to explain longitudinal variation in parasitemia of *Schellackia*. A final averaged model only included capture (importance = 1.00, $\beta = -1.37 \times 10^{-4} \pm 4.75 \times 10^{-5}$, $z = 2.88$, $p = 0.004$) (Supporting Information: Table S5). *Schellackia* infection significantly decreased between capture (mean ± SE parasites in 100 microscope fields = 11.80 ± 4.61) and recapture (5.25 ± 1.94) (Figure 3).

Model selection produced three models with AICc ≤ 2 to explain longitudinal variation in parasitemia of *Karyolysus*. A final model only included PC1_WC (importance = 1.00), with the estimated effect ($\beta = +1.19 \times 10^{-4} \pm 5.83 \times 10^{-5}$) having a moderate effect ($z = 2.04$, $p = 0.041$) (Supporting Information: Table S6). Thus, the infection by *Karyolysus* tended to be higher in those lizards with more total leukocytes, and more of both lymphocytes and monocytes. Noteworthy, the parasitemia of *Karyolysus* did not significantly change between capture (mean ± SE parasites in 100 microscope fields = 13.5 ± 1.97) and recapture (11.29 ± 1.83) (Figure 3).

4 | DISCUSSION

Capture–recapture studies are recommended over cross-sectional ones in investigations of host-parasite relationships (e.g., Sánchez et al., 2018). Such longitudinal studies performed on free-ranging hosts largely contribute to gain a comprehensive view of host-parasite relationships in nature. However, studies with these two characteristics are scarce. The low recapture rates (33% in our study) and high effort for implementing capture–mark–recapture studies are contributing factors limiting the number of longitudinal studies in the literature and, in turn, the same circumstance increases the value of the obtained data. We found positive associations between *Karyolysus* parasitemia and total leukocyte counts, lymphocytes, and monocytes, suggesting a significant immune activation associated with this parasite. However, the results indicated a greater recovery ability of the host against *Schellackia* infection, because the parasitemia of this latter parasite significantly decreased between capture and recapture, whereas that of *Karyolysus* did not. Parasites can evolve adaptive strategies to evade host defenses (Hisaeda et al., 2005; Schmid-Hempel, 2008, 2009). In this sense, *Karyolysus* blood stages are embedded in a parasitophorous vacuole (see Figure 3d), a structure that is not so obvious, perhaps absent, in *Schellackia* infections and that would confer them protection against host immune response (Beyer & Sidorenko, 1984). However, prevalence of both parasites remained similar after recapture,

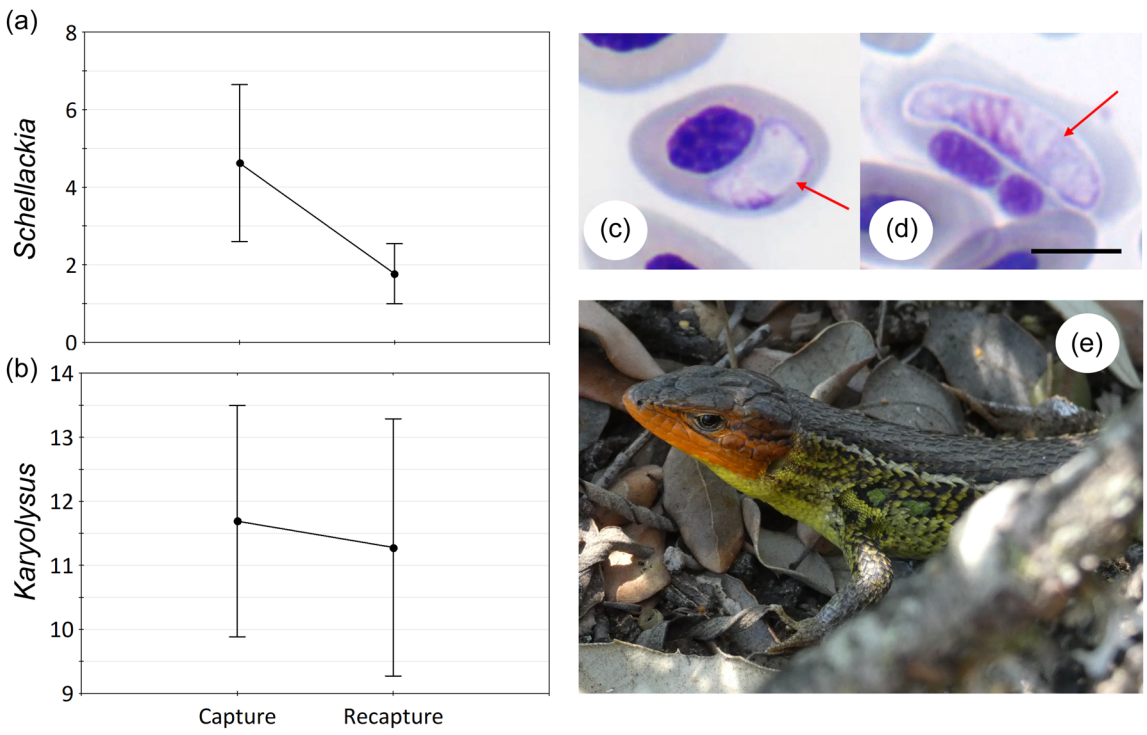

FIGURE 3 Within-lizard ($N = 39$) change in mean ± SE parasite raw counts of (a) *Schellackia* and (b) *Karyolysus* in 100 microscope fields. Microphotograph of (c) *Schellackia* and (d) *Karyolysus*. See the parasitophorous vacuole surrounding the parasite in (b) and its apparent absence in (a). Red arrows indicate the parasites within host erythrocytes and the scale bar indicates 5 μm. (e) Male of *P. algirus* with full development of its nuptial coloration.

indicating that lizards were unable to completely clear the parasites from their blood (Sorci, 1995).

No relationship was found between parasitemia of *Karyolysus* and nuptial coloration, travelled distance, or other parasites despite mites increased in translocated lizards and body condition decreased (see Barrientos & Megía-Palma, 2021). This is remarkable, because *Karyolysus* intensity tends to increase in correlation with male reproductive investment, as well as in ecological contexts of putative high competition and high environmental stress (Dajčman et al., 2022; Megía-Palma et al., 2016; Megía-Palma, Arregui, et al., 2020). This suggests that immune control of this parasite remains under equilibrium in lizards, unless energy is allocated to other physiologically demanding processes, such as reproduction (Megía-Palma, Merino, et al., 2022).

Less is known on the energetic costs and trade-offs associated with infection by *Schellackia* (e.g., Megía-Palma, Martínez, Merino, 2018). The significant reduction of this parasite between capture and recapture supports one of our initial predictions; lizards perform a better control of *Schellackia* infection. More opportunities to control the parasite replication might take place in the vertebrate, where all the reproductive stages of the parasite (asexual and sexual) occur (Telford, 2009). This would putatively confer a greater resistance against *Schellackia*, as *Karyolysus* can generate higher genetic diversity via genetic recombination during sexual reproduction in vectors. This hypothesis is further supported by the significantly lower prevalence of *Schellackia* in the sampled lizards in our study, despite mite vectors were present on 100% of recaptured lizards. This means that exposure to *Karyolysus* and *Schellackia* transmission is expected to be similar because mites of genus *Ophionyssus* are involved in the transmission of both blood parasites (Drechsler et al., 2021; Megía-Palma et al., 2023). Future molecular analyses on blood parasite prevalence in the mites will contribute to elucidate this supposition.

Our results suggest that the differential recovery ability from infections by *Karyolysus* and *Schellackia* is strongly influenced by co-adaptive histories of these parasites with the host *P. algirus* rather than by other concomitant effects like reproductive effort or putative sources of environmental stress (e.g., road proximity). Interestingly, no effects of body condition are observed, although the described effects of translocation on body condition of the lizards (Barrientos & Megía-Palma, 2021) and the putative effects of body condition on immunocompetence (e.g., Brown et al., 2023). The relationship between *Schellackia* and male nuptial coloration, therefore, conforms to expectations of the signaling theory; coloration would honestly reflect greater resistance against parasites in those host–parasite systems with closer co-evolutionary histories (Hamilton & Zuk, 1982). Thus, this view supports that *Schellackia*, a parasite with a closer biological and phylogenetic relationship with the Lacertidae (Megía-Palma, Martínez, Cuervo, et al., 2018; Megía-Palma et al., 2023), remains under a stronger immune control by the vertebrate host than *Karyolysus*.

Our results also indicate independent effects of the season and the manipulation on lizards' immune regulation. The negative relationship between capture date and PC1_WC suggests a down-regulation of total leukocyte count, lymphocytes, and monocytes, that has been previously associated with a seasonal increase in male reproductive effort in *P. algirus* (Puerta et al., 1996; Veiga et al., 1998). In contrast to this seasonal effect, we found an independent and positive relationship between PC1_WC and the number of days elapsed between capture and recapture. This suggests concomitant effects of handling on cellular components of the lizards' immune system, because monocytes and lymphocytes are, respectively, involved in both innate and adaptive immune response, and their abundance in peripheral blood is expected to increase should new infections occur (Cohn, 1968; Le Bien & Tedder, 2008). Therefore, the increase of these cellular components suggests an increase in the immune response mediated by lymphocytes and monocytes in the lizards after the initial manipulation, in turn suggesting that it would have also increased the lizards–pathogens contact rate. It is plausible that either blood extraction or the use of a contact thermometer (for purposes other than this study, e.g., Megía-Palma, Barja, et al., 2022) would have increased it. Similar conclusions were associated with the translocation experiment; lizards transported to the zone far from the road significantly increased total leukocyte counts, lymphocytes and monocytes as compared to control lizards. The relationship found suggests that infections increased in this specific group of lizards. Mites, *Karyolysus* and *Schellackia* all showed similar abundances between sampling zones in the initial sample of 120 males. Thus, elucidation of this effect only in the lizards that were translocated far from the road will require further investigation on a putative higher exposure to other pathogens (e.g., virus, bacteria, fungi) in this sampling zone.

In conclusion, our results show a differential recovery ability of lizards against *Karyolysus* and *Schellackia*. Unfortunately, the lack of identification of blood parasite genera in most of previous studies prevents us from accomplishing more precise comparisons. For this reason, we encourage future studies to identify the parasites investigated to provide a deeper understanding of host-parasite relationships in wild lizards.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Mendeley Data at https://data.mendeley.com/datasets/6xrv46rtxc/1.

ORCID

R. Megía-Palma http://orcid.org/0000-0003-1038-0468
R. Barrientos http://orcid.org/0000-0002-1677-3214

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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