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RESEARCH ARTICLE

Co-parasitism in the face of predation: Effects of natural enemies on a neotropical mockingbird

Jordan M. Herman[1] | **Vanina D. Fiorini**[2] | **Ignacio Crudele**[2] | **Juan C. Reboreda**[2] | **Shawn A. Pladas**[1,3] | **André P. Watson**[1] | **Sarah E. Bush**[1] | **Dale H. Clayton**[1]

[1]School of Biological Sciences, University of Utah, Salt Lake City, Utah, USA

[2]Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, IEGEBA-UBA-CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina

[3]Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana, USA

Correspondence
Jordan M. Herman
Email: jordan.m.her@gmail.com

Present address
Jordan M. Herman, HawkWatch International, Salt Lake City, UT, USA

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Abstract

1. Co-parasitism is ubiquitous and has important consequences for the ecology and evolution of wild host populations. Studies of parasite co-infections remain limited in scope, with few experimental tests of the fitness consequences of multiple parasites, especially in natural populations.
2. We measured the separate and combined effects of *Philornis seguyi* nest flies and shiny cowbirds *Molothrus bonariensis* on the fitness of a shared host, the chalk-browed mockingbird (*Mimus saturninus*) in Argentina.
3. Using a two-factor experimental approach, we manipulated the presence of nest flies and cowbirds in mockingbird nests and assessed their effects on mockingbird haemoglobin levels, begging and provisioning rates, body size, and fledging success. We also monitored rates of nest predation in relation to parasitism by flies and cowbirds.
4. Nest flies reduced the haemoglobin concentration, body size, and fledging success of mockingbirds, likely because mockingbirds did not compensate for parasitism by begging more or feeding their nestlings more. Cowbirds also reduced the fledging success of mockingbirds, even though they had no detectable effect on haemoglobin or body size. Nests with cowbirds, which beg more than mockingbirds, attracted more nest predators. There was no significant interaction between the effects of flies and cowbirds on any component of mockingbird fitness. The combined effects of nest flies and cowbirds were strictly additive.
5. In summary, we show that nest flies and cowbirds both reduce host fitness, but do not have interactive effects in co-parasitized nests. Our results further suggest that predators exacerbate the effects of nest flies and cowbirds on their hosts. Our study shows that the fitness consequences of co-parasitism are complex, especially in the context of community-level interactions.

KEYWORDS
brood parasitism, coinfection, community ecology, host–parasite interactions, natural enemies, nest predation, *Philornis* nest flies, shiny cowbirds

1 | INTRODUCTION

Many host species harbour diverse communities of parasites. Simultaneous infection by more than one parasite, which is the norm rather than the exception, is often associated with increased morbidity and mortality (Alizon et al., 2013; Pedersen & Fenton, 2015). However, the fitness consequences of co-occurring parasites can vary considerably among systems, ranging from harmful additive or synergistic effects to beneficial antagonistic effects (Graham, 2008; Vaumourin et al., 2015). This variation is often due to complex interactions between different kinds of parasites. For example, African buffalo (*Syncerus caffer*) parasitized by nematodes experience suppression of key immune defences against bacteria that cause bovine tuberculosis (Jolles et al., 2008). The nematodes and bacteria thus have a synergistic effect on host fitness: buffalo with tuberculosis are nine times more likely to die when co-parasitized by nematodes (Ezenwa et al., 2010). In contrast, antagonistic interactions between co-occurring parasites may benefit the host (Holt & Bonsall, 2017; Pedersen & Fenton, 2007). In waxworm caterpillars, for example, some bacterial strains produce toxins that kill other strains (Massey et al., 2004). Consequently, caterpillars coinfected with competing strains of bacteria have lower mortality than caterpillars infected with just a single strain. The nature and magnitude of the host–parasite and parasite–parasite interactions can have important implications for parasite virulence and transmission (Johnson & Hoverman, 2012; Sallinen et al., 2022), as well as host investment in defence and the susceptibility of the host to future infections (Jamieson et al., 2010; Telfer et al., 2010). In short, coinfection, or more broadly, co-parasitism, can have fundamental effects on the ecology and evolution of parasites and hosts.

Many studies of coinfection have focused on birds as hosts. Most bird studies have been surveys of co-occurring blood parasite species (Clark et al., 2016; Garcia-Longoria et al., 2022; Oakgrove et al., 2014; Santiago-Alarcon et al., 2011; Villalva-Pasillas et al., 2020). Other studies have been surveys of coinfection with different viruses (Agliani et al., 2023; Wille et al., 2015), viruses and blood parasites (Medeiros et al., 2014), or ectoparasites of different kinds (Sáez-Ventura et al., 2022). Mark-recapture methods have also been used to demonstrate negative correlations between blood parasite coinfections and the survival of birds in natural populations (Davidar & Morton, 2006; Marzal et al., 2008; Pigeault et al., 2018). However, in order to isolate the effects of co-parasitism on host fitness from the effects of other environmental factors, experimental manipulations of parasites are necessary (McCallum & Dobson, 1995). Few studies have experimentally manipulated one or both parasites of captive birds to assess the consequences of coinfection for host fitness (Dimitrov et al., 2015; Palinauskas et al., 2011, 2018, 2022; Reinoso-Pérez et al., 2020; Weitzman et al., 2020). Moreover, to our knowledge, there has been no study of the fitness consequences of experimentally manipulated coinfections of birds in natural populations. Here we report the results of a field experiment designed to measure the effects of co-parasitism on the reproductive success of wild birds. We explored the individual and combined effects of two common parasites, *Philornis* nest flies and brood-parasitic shiny cowbirds (*Molothrus bonariensis*), on the fitness of breeding chalk-browed mockingbirds (*Mimus saturninus*) in Argentina.

Parasitic nest flies are common parasites of birds in the neotropics. The genus *Philornis* includes about 50 known species of parasitic flies documented among the nests of more than 150 bird species (McNew & Clayton, 2018). Adult flies lay their eggs in bird nests, where they hatch into larvae and feed on the blood of nestlings, often leading to a reduction in haemoglobin (anaemia) and growth, followed by death (Arendt, 1985; Delannoy & Cruz, 1988; Knutie et al., 2016; Koop et al., 2011; Quiroga & Reboreda, 2012; Segura & Reboreda, 2011). In some cases, nestlings mitigate the effect of nest flies through increased begging, which can trigger increased provisioning (feeding) by parents, dubbed the "parental compensation hypothesis" (Bańbura et al., 2004; Christe et al., 1996; Norris et al., 2010; Richner et al., 1993; Williams & DeLeon, 2020). Several factors, such as resource availability, nestling size, and parental condition and investment (Hund et al., 2015; Knutie et al., 2016; Tomás et al., 2007; Wagner et al., 1997) may affect parental provisioning; however, more research is needed to fully understand the factors influencing compensatory feeding in response to parasitism.

Brood parasites are species that introduce their offspring into the nest or hive of an unrelated species, outsourcing the parental care of their offspring to the host. This form of parasitism has evolved repeatedly in unrelated groups of birds, insects, and fish (Pollock et al., 2021). Avian brood parasites are common nest parasites of breeding birds (Davies, 2000; Thorogood et al., 2019). In species where brood-parasitic nestlings are larger than host nestlings, the brood parasite can outcompete host nestlings for food. In such cases, brood parasite nestlings may grow faster and survive longer than host nestlings (Dearborn et al., 1998; Lichtenstein & Sealy, 1998). In other cases, such as shiny cowbirds in chalk-browed mockingbird nests, the mockingbird nestlings are larger than the cowbird nestlings and obtain an equal or majority share of food deliveries from the parents (Gloag, Tuero, et al., 2012; Tuero et al., 2016), which may help reduce the negative effect of the brood parasite on the growth and survival of host nestlings. Brood parasites could conceivably benefit larger host nestlings by stimulating increased parental provisioning, without monopolizing the additional food brought to the nest (Figure 1*D*; Bolopo et al., 2015; Gloag, Tuero, et al., 2012).

We experimentally manipulated the presence of nest flies and brood parasites in mockingbird nests to test for direct and combined effects of these parasites on several components of host reproductive success and behaviour, including haemoglobin level, the begging rate of nestlings, the rate of parental provisioning, and/or the body size and fledging success of nestlings (Figure 1). We also compared the predation rates of nests in relation to parasitism, since predation is one of the most common causes of nestling death in tropical environments (Husby, 2019; Tarwater et al., 2009). Moreover, indirect interactions between parasites and predators can substantially alter fitness consequences for shared victims (Hatcher et al., 2006,

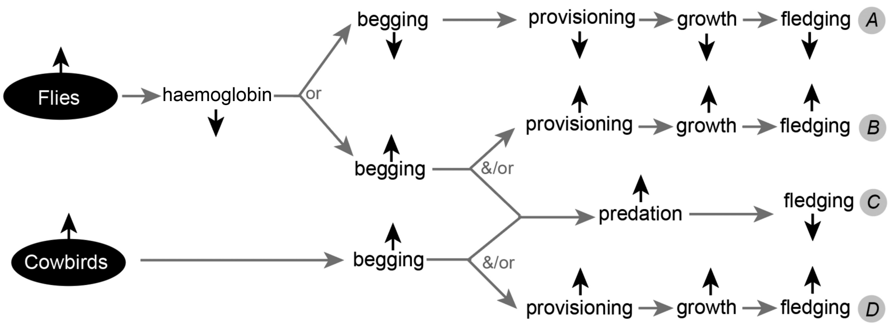

FIGURE 1 Components of mockingbird fitness predicted to change in response to experimental manipulation of nest flies or brood-parasitic cowbirds. 'Begging' is the mockingbird nestling begging rate. 'Provisioning' is the parental provisioning (feeding) rate. 'Growth' refers to increases in mockingbird nestling body mass, tarsus length, and/or primary feather length over time. 'Fledging' is mockingbird fledging success. (*A*–*D*) Letters to the right of each row correspond to descriptions in the text.

2012; Holt & Bonsall, 2017; Packer et al., 2003). For example, larval damselflies remove parasitic mites by vigorously rubbing their bodies, yet this grooming behaviour causes an increase in predation risk by acting as a visual cue to attract predatory fish (Rutherford et al., 2007).

In nests with flies, we predicted that a decrease in mockingbird nestling haemoglobin would cause mockingbird nestlings to beg less (Koop et al., 2011), leading to a decrease in parental provisioning, body size, and fledging success (Figure 1*A*). Alternatively, if the rate of begging increased in response to lower haemoglobin levels (Knutie et al., 2016), we predicted that provisioning, body size, and fledging success would increase, consistent with the parental compensation hypothesis (Figure 1*B*). We further predicted that increased begging would attract predators (Dearborn & Lichtenstein, 2002; Hannon et al., 2009; Ibáñez-Álamo et al., 2012; Massoni & Reboreda, 1998). Attraction of predators would lead to predation of nest contents and an abrupt reduction in fledging success (Figure 1*C*).

In nests with cowbirds, we predicted that an increase in total begging, due to the exaggerated begging of cowbird nestlings, would lead to an increase in parental provisioning, mockingbird body size, and mockingbird fledging success (Figure 1*D*). If increased begging also attracts predators, we predicted an increase in the rate of predation, with a concomitant reduction in fledging success (Figure 1*C*).

Flies and cowbirds could conceivably have interactive effects on mockingbird nestlings that go beyond their direct effects. For example, in nests co-parasitized by flies and cowbirds, a fly-mediated decrease in begging, due to anaemia (Figure 1*A*), could be offset by a cowbird-mediated increase in total begging and provisioning (Figure 1*D*). As another example, a fly-mediated increase in begging (Figure 1*B*), combined with begging by one or more cowbirds (Figure 1*D*), could increase begging enough to attract predators (Figure 1*C*). The potential for antagonistic or synergistic interactions between parasites means that a wide range of outcomes are possible in cases of co-parasitism by just two types of parasites.

2 | MATERIALS AND METHODS

The study was conducted over two breeding seasons (October–January 2017–2018 and 2018–2019) in the Reserva El Destino, Buenos Aires Province, Argentina (35°08′5.0316″ S, 57°23′30.3072″ W). This field site consists of flat pampas grassland interspersed with woodland patches and isolated trees, predominantly tala (*Celtis ehrenbergiana*), coronillo (*Scutia buxifolia*), and molle (*Schinus longifolius*).

2.1 | Mockingbirds

Chalk-browed mockingbirds are common at the site, where they establish territories in groups of small isolated shrubs or on the edge of adjacent woodlands. Nests are built 1–2 meters above ground and are large, open cup structures made from sticks and mud and lined with fine grass or horsehair (Fiorini et al., 2009b). The typical mockingbird clutch size is 3–4 eggs (Rabuffetti & Reboreda, 2007). Mockingbirds lay one egg per day, and females begin incubation with the penultimate egg. The incubation period lasts 13–14 days (Fiorini et al., 2009a). Mockingbird nestlings weigh about 6 g at hatching and are fed by both parents. They fledge 12–14 days after hatching, at which time they weigh 50–55 g (Fiorini & Reboreda, 2006).

We searched for active mockingbird nests by observing breeding pairs and using behavioural cues, such as foraging activity, male song, and aggression towards conspecifics and other bird species, to identify the approximate boundaries of each territory (Gloag et al., 2013). Nests were then located in the territory by systematically searching small trees and shrubs. Mockingbird pairs often re-nested in new nests within the same territory over the course of the breeding season but only had one active nest at a time. If a nest failed for any reason, we would search for new nests in the territory approximately 1 week later.

Nests were visited daily during the laying period. Once mockingbirds began incubating the eggs, we visited nests every 3–4 days early in the incubation period. Around the estimated hatch date of eggs, each nest was visited daily.

2.2 | *Philornis* nest flies

At our study site, *Philornis seguyi* is a common nest parasite that is found in up to 100% of mockingbird nests by the end of the breeding season (Rabuffetti & Reboreda, 2007). The larvae are subcutaneous and feed on nestling blood (Couri et al., 2005). Larvae develop in the host for 5–6 days, reaching a length of approximately 8–9 mm and a mass of 0.11–0.13 g. Once larval development is complete, the larvae exit the host's body and pupate in the nest material. Adult flies emerge from the pupae about 10 days later (Quiroga & Reboreda, 2013).

2.3 | Cowbirds

Shiny cowbirds are generalist brood parasites that exploit many host species. At our study site, shiny cowbirds are reproductively active throughout the mockingbird breeding season (Fiorini & Reboreda, 2006). A single mockingbird nest is often parasitized by more than one female cowbird during the egg-laying period. Before laying their own egg in the nest, cowbirds indiscriminately destroy existing mockingbird and/or cowbird eggs by pecking them (Fiorini et al., 2014; Gloag, Fiorini, et al., 2012). Mockingbird parents then remove punctured eggs from the nest. Mockingbird and cowbird nestlings that hatch from unpunctured eggs are reared together in mixed broods. Thus, mockingbird parents incur the energetic cost of rearing parasitic young in addition to the loss of their own punctured eggs. Shiny cowbirds have a slightly shorter incubation period than mockingbirds, such that cowbird nestlings usually hatch 1 day before their mockingbird nestmates. Cowbirds are considerably smaller than mockingbirds, weighing 3–4 g at hatching and 35–37 g at fledging (Fiorini et al., 2009a).

2.4 | Experimental design

We used a 2 × 2 factorial design to test for direct and combined effects of flies and cowbirds on mockingbird nestlings. Our design had four experimental treatments: (I) neither parasite, (II) flies, (III) cowbird, and (IV) both parasites (Figure 2). Treatment of the first nest was determined using a random number generator, with subsequent nests assigned treatments in a regular, alternating sequence.

Although many of the mockingbird pairs re-nested over the 4-month breeding season each year, we limited our analyses to the first reproductive attempt for each pair of birds because *Philornis* parasitism can have carry-over effects on parental reproductive investment in subsequent nesting attempts within years (McNew et al., 2020). Thus, subsequent nesting attempts are not independent of the experimental manipulations of first attempts. We considered a breeding pair's first reproductive attempt to be the first nest with hatched nestlings that was experimentally-manipulated.

2.5 | Experimental manipulation of nest flies

To quantify the effect of flies on host fitness, we experimentally eliminated flies by spraying half the nests with a 1% permethrin solution (Permectrin™ II). We sprayed the other half with water as a control (Figure 2). Nests were sprayed soon after the first nestling hatched, then again when nestlings were 4–5 days old. All nestlings and unhatched eggs were removed during the spraying process, then returned to the nest after it had dried (10–15 min). Our fumigation technique, which

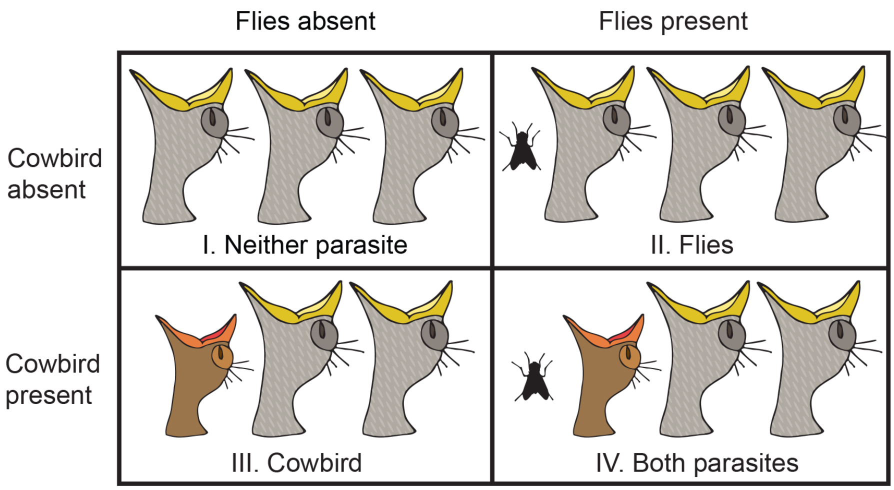

FIGURE 2 2 × 2 experimental manipulation of nest flies and shiny cowbird nestlings in chalk-browed mockingbird nests. Half of the nests contained three mockingbird nestlings (large grey heads); the other half contained two mockingbird nestlings and one cowbird nestling (small brown heads). Half the nests were sprayed with an insecticide to kill flies; the other half were sprayed with water as a control and were parasitized by flies. This two-factor design yielded four treatments: (I) neither parasite, (II) flies, (III) cowbird, and (IV) both parasites.

was based on previous studies (Knutie et al., 2016; Koop et al., 2013; McNew et al., 2019), has no apparent effect on nestlings.

To quantify mockingbird fly abundance (number of flies per nestling), we counted the number of larvae in each mockingbird nestling (Quiroga & Reboreda, 2012; Rabuffetti & Reboreda, 2007; Segura & Reboreda, 2011). *P. seguyi* larvae are subcutaneous, embedding themselves near the surface of the nestling's skin, where the caudal segment and spiracles of the larvae remain visible (Couri et al., 2005). Larvae were counted twice in quick succession. In the few cases when counts differed, this process was repeated until at least two counts matched.

2.6 | Experimental manipulation of cowbirds

To quantify the effect of shiny cowbirds on mockingbird fitness, we experimentally manipulated mockingbird clutches to mimic brood parasitism by female cowbirds. At our field site, the mean number of mockingbird nestlings in brood-parasitized nests at hatching is 1.7±0.2 (Fiorini, 2007). While mockingbird nests are parasitized by 1–2 cowbirds on average, our goal was to test the effects of just one cowbird nestling on mockingbird fitness. Thus, approximately half of the mockingbird nests were manipulated to contain two mockingbird eggs and one cowbird egg to simulate the replacement of a mockingbird egg by a cowbird egg (Figure 2). Mockingbird nests in the control treatment, i.e., without cowbirds, were manipulated to contain three mockingbird eggs. As in other studies, cowbird and mockingbird eggs were added or removed from nests prior to the onset of incubation (Fiorini & Reboreda, 2006). Unincubated mockingbird and cowbird eggs removed from nests were stored at room temperature (below 24°C) in a laboratory at our field site for a week or less. Unincubated eggs stored in this way remain viable upon incubation (Decuypere & Michels, 1992). Stored eggs were reallocated to other nests in the experiment, as needed. On average, cowbirds in our study hatched 0.5 days before mockingbirds, which is similar to the level of natural hatching asynchrony noted by Fiorini et al. (2009a) at our field site.

2.7 | Mockingbird nestling haemoglobin

At 8–9 days of age, nestlings were banded with a uniquely numbered metal band and their blood was sampled via brachial venipuncture. A small sample (<10 microliters) of blood was collected with a microcuvette and haemoglobin concentration (g/dL) was quantified immediately in the field using a HemoCue® Hb 201+ portable analyser.

2.8 | Nestling begging and adult provisioning behaviour

We used Yi Action Cameras and digital video recorders (Lawmate PV-1000 and Lawmate PV500 ECO) to film nestling begging and parental provisioning behaviour for approximately 3 h when nestlings were 4–5 days old. Nests were filmed between 07:30–14:30 h. Videos were analysed using the software BORIS (version 6.2; Friard & Gamba, 2016).

Nestling begging was defined as a nestling extending its neck with head raised and open mouth showing (Knutie et al., 2016; McNew et al., 2019). Total begging time was calculated as the percent of total video time during which at least one mockingbird or cowbird nestling was begging. Provisioning was measured as the average number of food items that were consumed by mockingbird nestlings per hour.

2.9 | Mockingbird body size

We considered day zero to be the day a nestling hatched. Morphological traits of mockingbirds (body mass, tarsus, and primary feather) were measured when nestlings were 0–1, 4–5, and 8–9 days old (McNew et al., 2019). Nestling age spanned 2 days due to asynchronous hatching. Nestlings were weighed to the nearest 0.1 g using a digital scale. Tarsus length and outermost (ninth) primary feather length were measured to the nearest 0.1 mm using digital callipers.

2.10 | Mockingbird predation and fledging success

During the 2018–2019 field season, we used time-lapse cameras to identify nest predators and further quantify the rate of nest predation. Once nestlings hatched, we suspended YI 4K Action and Sports Cameras (Yi Technology) within 30 cm of the nest. Cameras were powered by Anker PowerCore 13,000 mAh or 20,100 mAh portable chargers (Anker). Battery packs were connected to the cameras with three-meter-long USB cables hidden in the vegetation. We set cameras to take a photo every 30 s, creating a time-lapse record of nests during daylight hours. Because the cameras did not have infrared lights, it was not possible to record in the dark. We made daily visits to check nest contents and replace power banks to ensure continuous daytime surveillance. To minimize disturbance on days when we did not need to handle nestlings, we used the Yi Action Camera phone application to connect to the camera via Wi-Fi, enabling remote viewing of nest contents and time-lapse images. Reviewing time-lapse images allowed us to determine whether nestlings were removed by predators, or simply died in the nest. We considered nests to be preyed upon when a predator was captured on camera, or when nestlings disappeared from one frame to the next. By contrast, nestlings that died in the nest remained motionless for many frames. Nestlings that were in the nest for at least 13 days were considered to have successfully fledged.

2.11 | Statistical analysis

All analyses were conducted in RStudio (2021, R version 4.1.2) using the packages 'glmmTMB', 'lme4', 'emmeans', and 'multcomp.'

Degrees of freedom and *p*-values for all models were calculated using a Satterthwaite approximation with the 'lmerTest' package. Model validation was performed by visual inspection of residuals for all models.

Nest fly abundance was modelled using a zero-inflated generalized linear mixed model (GLMM) with a negative binomial error distribution. We included cowbird manipulation and year as fixed effects and nest ID as a random effect to control for the pseudoreplication of multiple nestlings per nest.

The effects of parasitism on nestling haemoglobin were analysed with a linear mixed model (LMM); the model included the fly manipulation, cowbird manipulation, their interaction, and year as fixed effects, and nest ID as a random effect.

The effects of parasitism on total begging time and provisioning rates of mockingbird nestlings were analysed with linear models (LMs) with fly manipulation, cowbird manipulation, their interaction, and year as fixed effects. The total begging time model also included number of nestlings as a fixed effect. Total begging was log-transformed for normality.

The effects of parasitism on the body size of mockingbird nestlings were analysed with LMMs, which included mockingbird nestling traits (body mass, tarsus, and ninth primary feather), fly manipulation, cowbird manipulation, nestling age, and year as fixed effects. We added a three-way interaction between fly manipulation, cowbird manipulation, and nestling age to test the separate and combined effects of the parasites on the nestlings over time. Nest ID was included as a random effect. Nestling primary feather lengths were square root-transformed to match modelling assumptions of linearity, normality of the residuals and homoscedasticity.

Nest predation rates were modelled with a generalized linear model (GLM) with a binomial distribution (1 = fledged, 0 = preyed upon). The GLM included fly manipulation, cowbird manipulation, and their interaction as fixed effects. This model did not include year because nest predation was only documented during the second field season (2018–2019).

Fledging success (proportion of nestlings that fledged) was modelled with a GLM with a binomial distribution. The response variable was a paired vector of the number of mockingbird nestlings that fledged versus the number of nestlings that died due to parasitism, predation, or unknown reasons for each nest. This approach was used because it accounts for differences in the number of mockingbird nestlings that hatched in nests among treatments (Crawley, 2012). The GLM included the fixed effects of fly manipulation, cowbird manipulation, their interaction, and year.

3 | RESULTS

A total of 75 nests were monitored over the two-year study: 37 nests in 2017–2018 and 38 nests in 2018–2019. These nests were fairly evenly distributed among the four experimental treatments (neither parasite $n = 16$; flies $n = 21$; cowbird $n = 20$, both parasites $n = 18$).

3.1 | Nest flies

Permethrin was very effective at killing fly larvae. The abundance of flies on mockingbird nestlings was (mean ± SE) 0.0 ± 0.0 for nests sprayed with permethrin ($n = 32$ nests) and 6.14 ± 0.96 for nests sprayed with water ($n = 37$ nests). Among the two treatments where flies were present (Figure 2), cowbirds had no significant effect on the abundance of flies (GLMM, $p = 0.23$, Table S1). The abundance of flies on mockingbird nestlings was 2.63 ± 0.61 in nests without cowbirds ($n = 34$ nests) and 3.92 ± 0.94 in nests with cowbirds ($n = 36$ nests). There was no significant difference in fly abundance between years (GLMM, $p = 0.13$, Table S1).

3.2 | Mockingbird haemoglobin

There was no significant interaction between flies and cowbirds on mockingbird nestling haemoglobin (LMM, $p = 0.13$, Table S2). Nest flies significantly lowered nestling haemoglobin (LMM, $p < 0.001$, Table S2; Figure S1), but cowbirds did not affect haemoglobin (LMM, $p = 0.18$, Table S2; Figure S1). Mockingbird haemoglobin values were significantly lower in the second year of the study (LMM, $p = 0.001$, Table S2), presumably because the humidity was higher that year (Herman, 2020); the HemoCue® devices used to measure haemoglobin are sensitive to high humidity (Whitehead et al., 2017).

3.3 | Nestling begging and adult provisioning behaviour

We quantified behaviour from 35 nests totalling 110 h of video over the 2-year study. Nests with a cowbird were excluded from behavioural analyses if the cowbird nestling was not alive at the time of filming. Additionally, some nests failed before nestlings were 4–5 days old and could not be filmed. There was no significant interaction between flies and cowbirds on total nestling begging time (mockingbird and cowbird nestlings combined; LM, $p = 0.48$, Table S3). Flies had no significant effect on total nestling begging time (LM, $p = 0.98$, Table S3; Figure 3A), but there was a significant increase in total nestling begging time of nests containing a cowbird (LM, $p < 0.001$, Table S3; Figure 3A). Total nestling begging time increased significantly with the number of nestlings, regardless of bird species (LM, $p = 0.02$, Table S3). Total nestling begging time did not differ significantly between years of the study (LM, $p = 0.87$, Table S3). When considering mockingbird nestlings alone, the amount of time that mockingbirds begged did not differ significantly among treatments (ANOVA df = 3,31, $F = 1.49$, $p = 0.24$; percent time (mean ± SE) mockingbirds spent begging per treatment: neither parasite: 5.46 ± 0.69, flies: 4.69 ± 0.84, cowbird: 4.36 ± 1.13, both parasites: 3.09 ± 0.47).

There was also no significant interaction between flies and cowbirds on the provisioning of mockingbird nestlings (LM, $p = 0.19$, Table S4). Flies had no significant effect on mockingbird provisioning rate (LM, $p = 0.28$, Table S4; Figure 3B), nor did cowbirds

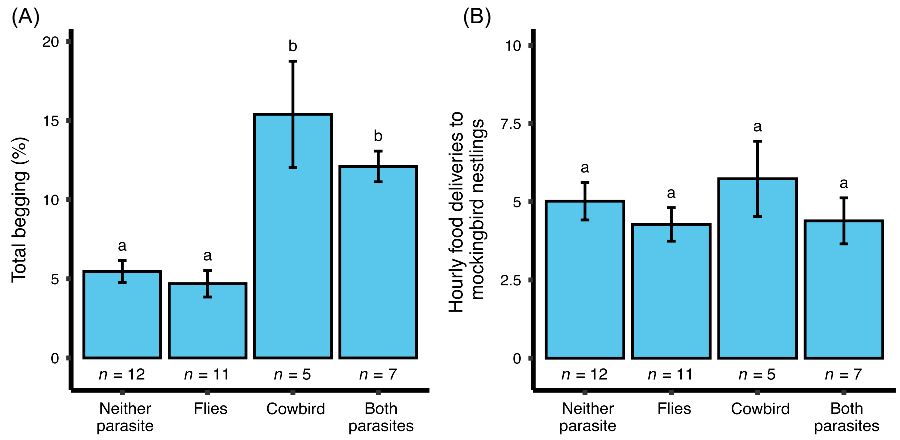

FIGURE 3 (A) Comparison of mean ± SE nestling begging time across four experimental treatments ($n = $ number of nests). Begging was significantly higher in the two treatments with cowbirds (different letters indicate significant differences among treatments, Tukey post hoc test, $p < 0.05$; see Table S3 for linear model [LM]). (B) Comparison of mean ± SE number of hourly food deliveries per mockingbird nestling across four experimental treatments. There was no significant difference in the provisioning of mockingbird nestlings, regardless of treatment (different letters indicate significant differences among treatments; Tukey post hoc test $p < 0.05$; see Table S4 for LM).

have a significant effect on provisioning (LM, $p = 0.40$, Table S4; Figure 3B). Adults provisioned mockingbird nestlings significantly more in the second year of the study (LM, $p < 0.01$, Table S4), perhaps because more rainfall that year resulted in more food resources (Herman, 2020).

3.4 | Mockingbird body size

The number of nests and nestlings included in analyses of mockingbird body size are reported in Table S5. Nestling mass and tarsus length did not vary with experimental manipulation at hatching, nor did primary feathers when they first emerged (flies, cowbird, flies:cowbird, LMM, $p > 0.05$ in all cases, Tables S6–S8). Mockingbird body mass, tarsus length, and ninth primary feather length increased significantly as the birds aged (Figure S2; LMM, $p < 0.001$ in all cases, Tables S6–S8).

There was no significant interaction between nest flies and cowbirds on mockingbird weight gain (LMM, flies:cowbird:age, $p = 0.12$, Table S6). Weight gain was not significantly influenced by flies (LMM, flies:age, $p = 0.05$, Table S6; Figure S2A) or cowbirds (LMM, cowbird:age, $p = 0.87$, Table S6; Figure S2B). Mockingbird body mass did not differ significantly between years of the study (LMM, $p = 0.78$, Table S6).

There was no significant interaction between nest flies and cowbirds on mockingbird tarsus length as nestlings aged (LMM, flies:cowbird:age, $p = 0.21$, Table S7). Tarsus length was significantly reduced by flies (LMM, flies:age, $p < 0.001$, Table S7; Figure S2C), but not cowbirds (LMM, cowbird:age, $p = 0.34$, Table S7; Figure S2D). Mockingbird tarsus length did not differ significantly between years of the study (LMM, $p = 0.26$, Table S7).

There was no significant interaction between nest flies and cowbirds on mockingbird feather length as nestlings aged (LMM, flies:cowbird:age, $p = 0.14$, Table S8). Feather length was significantly reduced by flies (LMM, flies:age, $p < 0.02$, Table S8; Figure S2E), but not cowbirds (LMM, cowbird:age, $p = 0.82$, Table S8; Figure S2F). Primary feather length was only measured in 2018–2019, so comparisons between years were not possible.

3.5 | Nest predation

A total of 31 nests were monitored for predation in 2018. Our cameras documented the following predators at mockingbird nests: crested caracara (*Caracara plancus*; Figure S3), roadside hawk (*Rupornis magnirostris*), chimango caracara (*Milvago chimango*), Argentine black and white tegu lizard (*Salvator merianae*), Patagonia green racer snake (*Philodryas patagoniensis*), and Geoffroy's cat (*Leopardus geoffroyi*; Figure S3). There was no significant interaction between flies and cowbirds on nest predation (GLM, $p = 0.38$, Table S9). Flies had no significant effect on nest predation (GLM, $p = 0.27$, Table S9): 58.3% ($n = 12$) of nests with flies were preyed upon compared to 47.4% ($n = 19$) of nests without flies. In contrast, cowbirds significantly increased nest predation (GLM, $p < 0.05$, Table S9). Indeed, this effect was quite large, 68.8% ($n = 16$) of nests with cowbirds were preyed upon, whereas only 33.3% ($n = 15$) of nests without cowbirds were preyed upon.

3.6 | Mockingbird fledging success

There was no significant interaction between flies and cowbirds on fledging success (GLM, $p = 0.15$, Table S10). Flies reduced mockingbird fledging success significantly (GLM, $p < 0.001$, Table S10; Figure 4). Cowbirds also reduced mockingbird fledging success significantly (GLM, $p = 0.001$, Table S10; Figure 4). Mockingbird fledging success did not differ between years of the study (GLM, $p = 0.10$, Table S10).

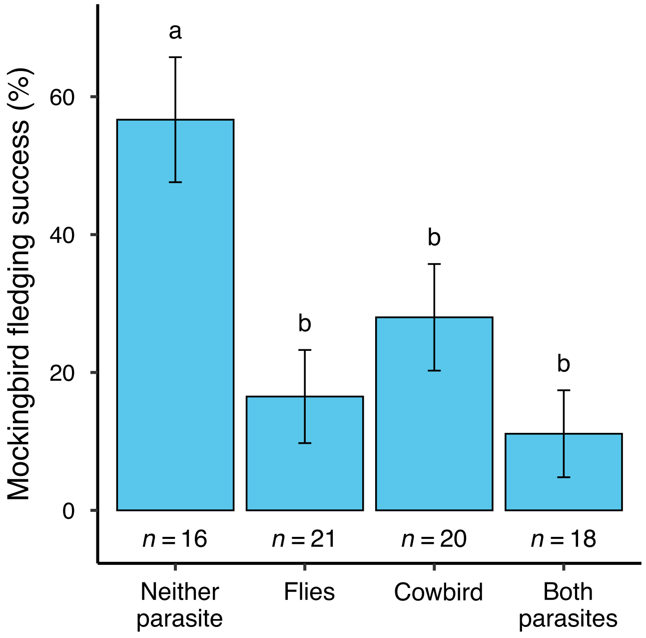

FIGURE 4 Mean ± SE mockingbird fledging success (%) in four experimental treatments (n = number of nests). Flies and cowbirds each had a significant negative effect on fledging success, but the interaction between parasite treatments was not significant (for generalized linear model see Table S10). Different letters indicate significant differences between treatments (Tukey post hoc test $p < 0.05$).

4 | DISCUSSION

In this study, we experimentally manipulated parasitic nest flies and brood-parasitic cowbirds in mockingbird nests in Argentina to investigate the separate and combined effects of parasites on several components of mockingbird fitness and behaviour. We also explored the potential influence of parasitism on nest predation.

Flies reduced the fledging success of mockingbirds in our experiment, thus confirming the observational results of Rabuffetti and Reboreda (2007) at the same location. The negative effect of flies on fledging success was likely due to anaemia and reduced body size. Mockingbird haemoglobin levels were significantly lower in nests with flies (Figure S1A). Mockingbirds also had shorter tarsi and primary feathers in nests with flies (Figure S2C,E).

Paradoxically, there was no significant effect of flies on the body mass of nestling mockingbirds (Figure S2A). This result is probably an artefact of the subcutaneous lifestyle of *P. seguyi* larvae, which makes it difficult to separate nestling mass from the mass of fly larvae growing beneath the skin of nestlings (Quiroga & Reboreda, 2013). Thus, any reduction in the body mass of parasitized nestlings may have been masked by the weight of the parasites themselves. Similarly, *P. trinitensis* parasites of tropical mockingbirds in Tobago, which are also subcutaneous, reduce tarsus growth but not the apparent body mass of nestlings (Knutie et al., 2017). By comparison, *P. downsi* larvae, which are not subcutaneous but live in the nest, do significantly reduce the body mass of Galápagos mockingbird nestlings (Knutie et al., 2016).

Cowbirds also reduced the fledging success of mockingbirds in our experiment, despite the fact that they did not cause anaemia or reduce body size. Rather, the impact of cowbirds was an indirect effect of increased nest predation. As predicted, the total begging time of nestlings increased when a cowbird was present: the total begging time of nests with cowbirds was more than twice that of nests without cowbirds (Figure 3A). Increased total begging was probably the cue used by predators in locating nests. This conclusion is consistent with other field studies showing that brood parasitism by brown-headed cowbirds (*Molothrus ater*) increased predation of their passerine hosts (Dearborn, 1999; Hannon et al., 2009), as well as a study showing that experimental addition of great spotted cuckoo (*Clamator glandarius*) brood parasites to common blackbird (*Turdus merula*) nests increases the rate of predation (Ibáñez-Álamo et al., 2012).

Mockingbird nestlings in nests with flies did not beg more, nor did their parents feed them more (Figure 3B) than in nests sprayed with permethrin to remove flies. The negative effect of flies on mockingbirds in our study was presumably partly a result of the lack of increased parental provisioning. By comparison, Galápagos mockingbird nestlings beg more in response to *Philornis* flies. Increased begging in this species stimulates the parents to feed the nestlings more, which compensates for the negative effect of the flies (Knutie et al., 2016). Why did the increase in total begging at nests with a cowbird not trigger increased parental provisioning, as in some other studies of brood parasites (Bolopo et al., 2015)? In several brood parasite–host systems, foster parents do not readily respond to exaggerated begging by parasitic nestlings, possibly because the hosts have evolved recognition and rejection behaviours of parasitic nestlings as a defence strategy (Soler, 2017). Insufficient food resources could also explain the lack of parental response to increases in nestling begging (Knutie et al., 2016). Previous research found that mockingbird parents do not compensate for the effects of parasitism on nestlings during drought years when food is scarce (McNew et al., 2019). However, our study was conducted during two seasons of regular rainfall, suggesting that the impact of flies on chalk-browed mockingbirds is unlikely to be due to food scarcity. To test this hypothesis, future studies could quantify or manipulate the abundance of food resources in the environment and compare these measurements to parental feeding rates, fly parasitism, and nestling fitness.

A more likely explanation, perhaps, for the difference in the begging and provisioning responses of chalk-browed and Galapagos mockingbirds is a differential risk of nest predation. Generally speaking, birds breeding in areas with high nest predation rates are known to beg less than birds in areas with low predation rates to minimize detection due to begging (Briskie et al., 1999). In experiments where recorded begging calls were played at artificial nests containing eggs, a higher number of eggs were taken from noisy nests compared to silent control nests (Haskell, 1994; Leech & Leonard, 1997). More frequent provisioning trips are also associated with increased nest predation (Fontaine & Martin, 2006; Lima, 2009; Martin et al., 2000). Nest predators in the Galápagos are rare (Grant & Grant, 2008), which may explain why Galápagos mockingbird nestlings readily increase their rate of begging in response to *P. downsi*. In other words, our data suggest that mockingbirds may experience a trade-off between anti-predator and anti-parasite defences. This hypothesis is consistent with experimental and theoretical studies showing that host behavioural defences against parasites are constrained by predators in the community (Friman & Buckling, 2012; Toor & Best, 2016). A more definitive test of this hypothesis will require a comparison of nest predation rates between nests with experimentally-manipulated levels of mockingbird nestling begging.

Our experiment further showed that the effects of nest flies and cowbirds on chalk-browed mockingbirds were additive, not synergistic. There were no positive or negative interactions between cowbirds and flies on any measure of mockingbird fitness. The additive effects of both parasites reduced mockingbird fitness, but in different ways. Nest flies reduced haemoglobin levels and body size, both of which presumably contributed to the lower fledging success of mockingbirds (Figure 4). Cowbirds increased total begging time, which presumably contributed to the increase in nest predation and thus lower fledging success (Figures 3 and 4). Neither parasite mediated, nor exacerbated, the effect of the other.

Our results contrast with those of Smith (1968), who reported that giant cowbird (*Molothrus oryzivorus*) nestlings remove *Philornis* fly eggs and larvae from oropendola and cacique host nestlings (*Psarocolius* spp. and *Cacicus cela*) by allopreening them. Smith further reported that the fledging success of hosts was increased as a consequence of this interaction between the cowbirds and nest flies. Thus, the interaction between the two parasites is an example of antagonistic co-parasitism. Smith's study, which has been questioned, still requires independent confirmation (Bush & Clayton, 2018).

In summary, our study shows that nest flies and cowbirds each reduce the fitness of their shared host, and that their combined effects are additive. While we did not detect a significant interaction between flies and cowbirds on mockingbird fitness in co-parasitized nests, our data suggest that ecological interactions with other members of the community (predators) indirectly influenced the effects of parasitism on the host. In short, our study shows that the fitness consequences of co-parasitism are complex, especially in the context of broader community-level interactions.

AUTHOR CONTRIBUTIONS

Jordan M. Herman, Dale H. Clayton, Sarah E. Bush, Vanina D. Fiorini, and Juan C. Reboreda conceived the ideas and designed methodology. Jordan M. Herman, Ignacio Crudele, Shawn A. Pladas, and Andre P. Watson collected the data. Jordan M. Herman analysed the data. Jordan M. Herman, Dale H. Clayton, Sarah E. Bush, and Vanina D. Fiorini led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.sn02v6x4n (Herman et al., 2023).

ORCID

Jordan M. Herman https://orcid.org/0000-0003-1377-5844
Vanina D. Fiorini https://orcid.org/0000-0003-0447-461X
Ignacio Crudele https://orcid.org/0000-0002-6861-9008
Juan C. Reboreda https://orcid.org/0000-0001-5136-4574
Sarah E. Bush https://orcid.org/0000-0002-2913-4876
Dale H. Clayton https://orcid.org/0000-0003-1698-3542

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SUPPORTING INFORMATION

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

Figure S1. Mean ± SE mockingbird nestling hemoglobin levels at 8–9 days old when (A) flies were absent or present, and (B) cowbirds were absent or present.

Figure S2. Mockingbird body mass (A, B), tarsus length (C, D), and primary feather length (E, F) in relation to fly and cowbird experimental manipulations.

Figure S3. Examples of nest predators captured on camera; left: southern crested caracara caracara (*Caracara plancus*); right: melanistic Geoffroy's cat (*Leopardus geoffroyi*).

Table S1. Zero-inflated generalized linear mixed-effects model of the effects of cowbird nestlings and year on mockingbird nestling fly abundance (number of flies per mockingbird nestling).

Table S2. Linear model of the effects of experimental manipulations and year on mockingbird nestling hemoglobin concentration.
Table S3. Linear model of the effects of experimental manipulations, the number of nestlings, and year on total nestling begging time (mockingbird and cowbird nestlings combined).
Table S4. Linear model of the effects of experimental manipulations, their interaction, and year on parental provisioning (average hourly rate of food deliveries to mockingbird nestlings).
Table S5. Summary of the number of nests (nestlings) in the statistical analyses of mockingbird nestling growth (body mass, tarsus, and ninth primary feather).
Table S6. Linear model of the effects of experimental manipulations, age, and year on mockingbird nestling body mass.
Table S7. Linear model of the effects of experimental manipulation, age, and year on mockingbird nestling tarsus length.
Table S8. Linear model of the effects of experimental manipulation and age on mockingbird nestling ninth primary feather length.
Table S9. Generalized linear model of the effects of experimental manipulations on nest predation (1 = preyed upon, 0 = fledged).
Table S10. Generalized linear model of the effects of experimental manipulations and year on fledging success (proportion of nestlings that fledged).

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