Darwin’s finches habitually anoint their feathers with leaves of the endemic tree *Psidium galapageium* during the non-breeding season

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**Abstract**
Birds host a wide range of ectoparasites and have developed behavioural strategies to combat them, such as preening, dust bathing and water bathing. In addition, a wide range of avian taxa anoint their feathers with insects or plants that have pharmaceutical properties, though most observations on anointing are anecdotal. Darwin’s finches preen with leaves of an endemic tree (*Psidium galapageium*) and a previous laboratory study has shown that this plant has compounds that repel both mosquitoes and the invasive parasitic fly *Philornis downsi*, whose larvae suck blood from nestlings and incubating females and cause high nestling mortality. In the current study, we tested the hypothesis that preening with *P. galapageium* leaves serves to repel these parasites with an indirect approach. Mosquitoes and *P. downsi* affect their hosts mainly during the bird breeding season and *P. downsi* only affects breeding females, but not adult males. To test our hypothesis, we gathered quantitative data on leaf-preening behaviour in Darwin’s finches during their breeding and non-breeding season and also investigated the influence of time of day and humidity, as humid conditions facilitate the release of volatile organic compounds. Contrary to our predictions, anointing occurred significantly more often during the non-breeding season when mosquito and *P. downsi* numbers are lower. Four Darwin’s finch species anointed their feathers habitually, and during the non-breeding season, 56% of all preening events were with leaves. We found no effect of sex, but preening with leaves occurred predominately in the morning when leaves were wet. Our study is the first to provide quantitative data on anointing behaviour in birds and the high percentage of preening with leaves in the non-breeding season suggests that the behaviour has an adaptive value. However, further studies are needed to test whether it reduces the negative impact of parasites other than mosquitoes and *P. downsi*.

**KEYWORDS**
Darwin’s finches, *Philornis downsi*, preening, *Psidium galapageium*, self-medication
Parasites can have strong detrimental effects on host fitness and are therefore a major selective force on various host traits (Clayton & Moore, 1997; Grenfell et al., 1995; Loye & Zuk, 1991; Rätti et al., 1993; Toft et al., 1991). Hosts have developed a wide array of defence mechanisms to counteract the effects of parasitic pressure on their fitness (reviewed in Clayton & Wolfe, 1993). Birds are hosts to various types of ectoparasites including arthropods, bacteria and fungi (reviewed in Bush & Clayton, 2018). To combat them, they have developed a range of nest and body maintenance behaviours such as nest sanitation, preening, allopreening, scratching, water bathing, dust bathing and sunning (reviewed in Bush & Clayton, 2018). In addition, a wide range of avian taxa supplement their behavioural strategies with self-fumigation, which is the application of substances to the body or their nests for the treatment or control of parasites or parasite vectors (reviewed in Bush & Clayton, 2018; Huffman, 2019). These substances include insects, plants or even human-made products, which may be pungent and/or have pharmaceutical properties. For instance, common starlings (Sturnus vulgaris) and Eurasian blue tits (Cyanistes caerulescens) incorporate specific fresh herbs with antimicrobial properties into their nests (Costa-Neto, 2012; Gwinner, 2013; Lambrechts & Dos Santos, 2000), while house sparrows (Passer domesticus) and house finches (Carpodacus mexicanus) incorporate the fibres of cigarette butts into their nests (Suárez-Rodríguez et al., 2013). A taxonomically more widespread behaviour is the anointment of feathers and skin with insects (e.g. ants, millipedes; Ehrlich et al., 1986; Parkes et al., 2003) or aromatic plants (e.g. Maia & Moore, 2011; Moore et al., 2006; Weldon et al., 2011). “Anting” is a behaviour which involves the anointment of the feathers with ants and is performed habitually by over 200 bird species (Clayton et al., 2010). The formic acid that is released during anting is assumed to repel harmful parasites (Falótico et al., 2007; reviewed in Potter, 1970; Revis & Waller, 2004). Anointing with plant materials has been observed in only a few bird species and observations are rare or anecdotal (reviewed in Clayton & Wolfe, 1993). For instance, common grackles (Quiscalus quiscula) have been observed to preen with marigold flowers (genus Tagetes), which contain a natural insect repellent (Nero & Hatch, 1984), as well as with the pulp and rind of a lime fruit, which have insecticidal properties (Clayton & Vernon, 1993; Rodríguez & Wrangham, 1993). Additionally, the monarch flycatchers (Chasiempis sandwichensis) rub berries of Brazilian pepper (Schinus terebinthifolius), which has antibiotic properties, onto their feathers (VanderWerf, 2005).

The current study investigated anointing behaviour in Darwin’s finches. In 2012, a green warbler finch (Certhidea olivacea) and later four other species of Darwin’s finches were observed for the first time tearing off the leaves of the endemic tree Psidium galapageium and rubbing them onto their feathers (Cimadom et al., 2016). The authors observed two different methods of anointing: 1) the sponge method, in which the bird threads a piece of leaf through its feathers and 2) the lotion method, in which the bird chews the leaf first and applies the mashed leaf to its feathers. Cimadom et al., (2016) hypothesised that the birds use these leaves to repel ectoparasites that negatively impact the fitness of Darwin’s finches, namely the blood-sucking fly Philornis downsi and mosquitoes. Several mosquito species are native to the Galápagos Islands but others have been introduced (e.g. Culex quinquefasciatus, Aedes aegypti; Sinclair, 2013) and transmit novel mosquito-borne pathogens such as avian poxvirus (Parker et al., 2011). The avian poxvirus causes lesions on toes, legs, and the tissue around the bill. Individuals that survive often have deformed or missing digits (Parker et al., 2011). The introduced parasitic fly P. downsi has an even stronger effect on the fitness of Darwin’s finches than the poxvirus: approximately 55% of Darwin’s finch nestlings die annually due to parasitism by this species (Fessl et al., 2018; Kleindorfer & Dudaniec, 2016). The fly’s first larval stage is mainly found in the nestlings’ nostrils. The second and third larval stages live in the bottom of the nest where they penetrate the skin of the nestlings and consume their blood (Fessl et al., 2006) and also attack incubating females (Cimadom et al., 2016; Knutle et al., 2013). Cimadom et al., (2016) demonstrated a repellent effect of extracts of P. galapageium on mosquitoes and adult P. downsi and a growth inhibiting effect on P. downsi larvae in the laboratory. The abundance of the above-mentioned parasites varies seasonally. P. downsi affects Darwin’s finches mainly during the birds’ breeding season (January – April), which is when their parasitic larval stage occurs in the finches’ nests (Fessl et al., 2006). Mosquito abundance is also higher during the warm and rainy breeding season, as it is only then that favourable temperatures (> 20°C) and the availability of stagnant water pools stimulate mosquito breeding (Asigau et al., 2017; Khan et al., 2018). On the Galápagos Islands, mosquito abundance increases with precipitation but decreases with altitude (Asigau & Parker, 2018; Bataille et al., 2010). Combined, these factors result in very low abundance of mosquitoes in the highlands of the Galápagos Islands during the cool non-breeding season (Asigau et al., 2017). The seasonality of P. downsi and of mosquitoes allows for an indirect approach to test whether Darwin’s finches use P. galapageium leaves to repel them. Here, we chose to concentrate on these parasites although Darwin’s finches also suffer from other parasites, such as feather mites and feather lice (Palma & Peck, 2013; Villa et al., 2013). If P. downsi and mosquitoes are targeted by this behaviour, the frequency of preening with leaves should also match the abundance of the targeted organisms.

Furthermore, it has been suggested that time of day and humidity have an effect on preening behaviour and could also influence preening with plants that release volatiles. Wet conditions of high humidity are known to facilitate the release of volatile organic compounds (VOC; Gouinguené & Turlings, 2002; Salerno et al., 2017; Vallat et al., 2005). In several bird species, the frequency of preening peaked in the early morning (Henson et al., 2007; Robbins, 1981) and increased with increasing humidity (Brown, 1974; Henson et al., 2007).

In our study, we tested the hypothesis that preening with leaves by Darwin’s finches serves to repel the invasive parasitic fly P. downsi and/or native and introduced mosquitoes. We predicted that if preening with P. galapageium serves to protect incubating females
from *P. downsi*, the behaviour should be more frequently observed in females and more frequently during their breeding season. If preening serves to protect against mosquitoes, we expected the behaviour to be evenly distributed across both sexes and to increase with the high abundance of mosquitoes in the breeding season. To test these hypotheses, we gathered quantitative data on leaf-preening behaviour of Darwin’s finches during the breeding and non-breeding season. We measured whether the occurrence of leaf-preening is influenced by season, time of day or wetness of leaves and whether it differs between the sexes.

2 | METHODS

2.1 | Study area

This study was conducted in Los Gemelos (0°37'34” S, 90°23'10” W), at an elevation of around 600 m, in the humid “Scalesia” forest on the Island of Santa Cruz, Galápagos from January 19–March 8, 2019 (main breeding season of Galápagos landbirds) and September 3–25, 2019 (non-breeding season). Some of the most prominent tree species in the forest are the endemic species *Scalesia pedunculata*, *Solanum cheesmaniae* and *Psidium galapageium*. The forest has been invaded by alien shrubs such as *Rubus niveus*, *Tradescantia fluminensis* and *Cestrum auriculatum* (Rivas-Torres et al., 2018).

The climate on the Galápagos Islands is highly seasonal. During the breeding season, from January to April, mean air temperatures in the humid highlands range between 16 and 28°C and the skies are usually clear with occasional heavy rain showers. During the non-breeding season from June to December, the temperature is lower (ranging between 13 and 20°C) and while there is hardly any precipitation in the lowlands, the highlands are continuously wet due to a consistent, dense mist (Causton et al., 2019; Jackson, 1993).

2.2 | Behavioural observations

We selected 30 points for behavioural observations. Each point contained at least three medium-sized *Ps. galapageium* trees (>3 m high) and points were 100 m apart from each other (Figure S1). The distance between observation points was chosen to minimise possible overlaps of bird territories between points and was measured via GPS. At each point, we recorded the following habitat parameters: canopy height as well as the total number of and height of *Ps. galapageium* within a radius of 15 m (hereafter “point radius”). The point radius was measured with a laser range finder. Each point was visited five times per season between 6:00 and 11:00, which is the period of highest bird activity. On average, eight points were visited per day and routes between points were chosen so that each point was visited at different hours of the day. At each point, all behavioural observations were made within the 15 m point radius. After an initial training phase involving two observers (ST and TS), all behavioural observations were made by one person (TS). At the beginning of each visit, the observer noted the number and species identity of the passerines present within the point radius. Then, for a duration of 30 min, preening events were recorded, along with the specification of whether preening was conducted with or without *P. galapageium*. The observer did not record whether birds that preened with leaves used the sponge or lotion method because this would have required focal observation of preening individuals and would have increased the probability of missing preening individuals. The following parameters were recorded for each preening individual: species identity, life stage (adult vs juvenile) and sex (male vs female/immature male combined; see below for explanation of latter categorization). Adult males were identified using plumage characteristics, beak colour and song activity: small ground finch (*G. fuliginosa*), medium ground finch (*G. fortis*) and large ground finch males (*G. magnirostris*) show a streaky plumage from crown to chin and their overall plumage darkens with every moult until they are completely black (Kleindorfer et al., 2019). Small tree finch (*Camarhynchus parvulus*) females and immature males have a light brown colouration. Mature males have black feathers on the head, starting at the beak and later forming a dark hood that extends down to throat and breast (Kleindorfer et al., 2019). Green warbler finch females and immature males have a grey-greenish colouration while males can be distinguished by their orange throat (Kleindorfer et al., 2019). In Darwin’s finches, only males sing, thus any bird that sang was identified as male. However, yearling males of all Darwin’s finches that do not sing cannot be distinguished from females and were therefore categorised as being in the female-immature male group (other). Juveniles were identified by pink beak colouration and begging behaviour. We distinguished individuals that were present at the same time and new arrivals based on species, sex and plumage colouration. Observations of individuals that looked the same and were not observed simultaneously were excluded from the data set.

At the start of each observation, leaves of *P. galapageium* at the observation point were scored as wet when the leaf surface exhibited visible dew or water droplets. Otherwise, they were scored as dry. The initial time was noted as well as the weather conditions, which were categorised as rain versus no rain and sun versus fog. Temperature and humidity were recorded with two DS1923 hygro-chron temperature/humidity data loggers (iButton®).

2.3 | Statistical analyses

For the statistical analyses, juveniles were excluded because they were not observed preening with leaves during the breeding season. Only data from four species (the green warbler finch, small ground finch, medium ground finch and small tree finch) were analysed. Observations of the woodpecker finch (*C. pallidus*), large tree finch (*C. psittacula*), Galápagos flycatcher (*Myiarchus magnirostris*) and yellow warbler (*Setophaga petechial aureola*) were excluded because they were never or only once observed preening with leaves (Table 1).
was done using the package \texttt{lme4} \citep{Bates2015}. Data and code are available online (https://doi.org/10.17605/OSF.IO/4P75C, Tebbich et al., 2020).

We fitted a generalised linear mixed regression model to the binary preening data (with leaves versus without leaves) to test for effects of season (non-breeding versus breeding season), sex (male versus other), wetness of leaves (dry versus wet), and time of day. Season, sex, and wetness of leaves were included as binary categorical predictors and time was treated as a numerical predictor (time of day at preening event, ranging from 6:00 to 11:00). To account for the fact that the observed preening events varied substantially between observation points, we included point ID, ranging from one to 30, as a random factor (random intercept) in our model. The statistical significance of coefficients was assessed using likelihood ratio tests.

We had no clear a priori hypotheses concerning which species might anoint with \textit{P. galapageium} more or less than the others nor concerning how rain, sun or temperature would affect preening behaviour. We did not conduct hypothesis tests for these variables and did not include them in the regression model, but we explored their effects by visualising their associations with preening.

All statistical analyses were conducted in the statistical programming language R (version 4.0.1, R Core Team, 2020). R-package \textit{janitor} \citep{Firke2020} was used for data cleaning, package collection \textit{tidyverse} \citep{Wickham2019} and package \textit{patchwork} \citep{Pedersen2020} were used for analysis and plotting. Model fitting was done using the package \textit{lme4} \citep{Bates2015}. Data and code are available online (https://doi.org/10.17605/OSF.IO/4P75C, Tebbich et al., 2020).

### 3 | RESULTS

We observed 229 preening events in total for the four species included in the analysis (the green warbler finch, small ground finch, medium ground finch and small tree finch). Of these observations, 146 were without leaves and 83 were with leaves.

The average temperature calculated over all preening events was 15.4°C (STD = 1.12) in the non-breeding season and 20.8°C (STD = 3.82) in the breeding season. It rained in 76% of all observations in the non-breeding season but only in 6% of all observations in the breeding season. The frequency of observations with sunshine was 2% in the non-breeding season and 52% in the breeding season.

The average humidity was 99.9% (STD = 0.98) in the non-breeding season and 96.5% (STD = 5.50) in the breeding season.

Preening with leaves occurred predominantly in the non-breeding season and peaked in the early morning hours in all four observed species. Preening without leaves was more evenly distributed across the morning and occurred mainly in the breeding season (Figure 1a, b). In the non-breeding season, 56% of all preening events were with leaves, whereas in the breeding season only 10.8% were with leaves.

The effects of season and time of day on the probability of preening with leaves were statistically significant ($p < .001$ for both). Effect size estimates derived from the model for season and time of day were similar with and without inclusion of sex and wetness of leaves as predictors. The estimated probabilities of preening with leaves in the breeding versus non-breeding season were 0.36 and 0.85 at 6:00; however, at 10:00, they were 0.01 and 0.09, respectively.

Both sexes preened with leaves at a similar frequency (effect of sex was not statistically significant in the model, $p = .16$; Figure 2).

There were species differences in the preening patterns between the seasons (Figure 1a). For example, medium ground finches preened predominantly in the non-breeding season and almost exclusively with leaves, whereas warbler finches preened mainly without leaves in the breeding season.

The behaviour of preening with leaves occurred when wet leaves were present in the non-breeding season (Figure 3) and both preening with leaves and occurrence of wet leaves showed the same decreasing daily trend (Figure 4). However, the effect of leaf wetness on preening with versus without leaves was strongly correlated with season ($r = 0.66$) and therefore was itself not statistically significant in the model ($p = .10$). Humid conditions were ever-present in the non-breeding season, so the vegetation was frequently wet (correlation between rain and presence of wet leaves was $r = 0.77$, correlation between season and rain was $r = 0.70$).

### 4 | DISCUSSION

Contrary to our prediction, leaf preening occurred more frequently in the non-breeding season, when mosquito activity is low and
Darwin’s finches are not affected by *P. downsi* larvae. In addition, adult males preened as often as females and immature males. These findings suggest that preening with *P. galapageium* leaves does not primarily target mosquitoes and *P. downsi* larvae, as mosquitoes and *P. downsi* are not abundant during the non-breeding season and only females are affected by the blood-sucking larvae of *P. downsi*.

The behaviour could, however, be important for repelling other ectoparasites such as feather mites, feather lice, feather-degrading bacteria and fungi. Villa et al., 2013 recorded 8 genera of feather mites from the same Darwin’s finch species in the same study area during the breeding season. Seasonal patterns of their diversity, prevalence and intensity have not been studied to date. An experimental study on feather mites demonstrated that mites suffer from desiccation at relative humidity (RH) below 55% (Gaede & Knülle, 1987); however, in our study area, the RH was always well above this value in both seasons. In addition, most feather mites do not negatively impact birds (Dowling et al., 2001; Matthews et al., 2018), rather they have a mutualistic relationship with their host, cleansing the birds’ feathers from fungi and bacteria (Doña et al., 2019). Villa et al., (2013) did find very small numbers of blood-feeding mites (*Pellonyssus* sp.) on Darwin’s finches, but these numbers were too low to enable investigation of seasonal patterns. Given the small numbers found on Darwin’s finches, it does not seem likely that the blood-feeding mites are the main target of leaf-preening behaviour.

In addition to mites, three genera of lice have also been found on Darwin’s finches (*Brueelia*, *Myrsidea* and *Philopterus*; Palma & Peck, 2013). The feather louse (*Myrsidea rustica*) has indirect negative effects on flight performance of barn swallows (*Hirundo rustica*; Barbosa et al., 2002) and lice load increased with humidity in mourning doves (*Zenaida macroura*) and Inca doves (*Columbina inca*; Moyer et al., 2002). However, dust ruffling of 26 Darwin’s finches during the non-breeding season in 2020 revealed low prevalence (30.8% of birds with lice) and very low intensity (mean 2.0 ± SE 0.42 lice per infested bird; *n* = 8) of feather lice, making them an unlikely target (Courtney Pike unpublished data).

Keratinophilic fungi or feather-degrading bacteria have not yet been studied in Darwin’s finches but are found in a wide range of avian taxa. They have been associated with impaired flight performance...
and an increased risk of predation by raptors (Al Rubaiee et al., 2017; Møller et al., 2012). In the northern United States, incidences of feather bacteria on house sparrows were highest in late fall and winter (Burtt & Ichida, 1999). The authors suggest that the higher incidence may be related to higher temperature and humidity but also to increased exposure to ultraviolet (UV) light, as it kills the vegetative cells of bacteria and their spores (Madigan et al., 1997). Humidity was always high at our study site, but temperature and the number of hours of sunshine were low during the non-breeding season. This combination of environmental factors could potentially lead to seasonality in feather-degrading bacteria. The influence of climate conditions on feather-degrading fungi is less clear. A study in house sparrows (*Passer domesticus L*.) showed no overall seasonal pattern (Hubálek, 1976).

Analysis of the ethanolic extract and the essential oil of *P. galapageium* and its respective fractions (Cimadom et al., 2016) revealed that this plant not only contains insect repellent or insecticidal compounds but also compounds with antimicrobial properties (α-Pinene, Nerolidol, Eucalyptol, Terpinene, Guiaol; Chan et al., 2016; Choudhary et al., 2007; Gilles et al., 2010; Iacobellis et al., 2005; Maciel et al., 2010; Nissen et al., 2010). In addition, the closely related tree species, *Psidium guajava*, has antifungal and antibacterial properties (Morais-Braga et al., 2017; Padrón-Márquez et al., 2012; Pandey & Shweta, 2011). Combined, these findings raise the possibility that preening with *P. galapageium* reduces feather-damaging microbes. However, this needs to be tested in future studies with an experimental approach in which the incidence and seasonality of feather microbes is sampled. The antimicrobial properties of *P. galapageium* could be tested in vitro on the samples taken from the birds’ feathers. *P. galapageium* also contains eucalyptol (Cimadom et al., 2016, Martina et al. submitted), which has skin soothing properties (Gilles et al., 2010). If parasites cause itchy skin irritations, a soothing effect of *P. galapageium* could elicit the behaviour of preening with leaves.

As predicted, the behaviour of preening with leaves co-occurred with the presence of wet leaves in the non-breeding season. During the breeding season, almost all of the few observations of preening with leaves occurred before 9:00. Both humidity and moistness of leaves decreased with time of day, which could explain why preening with leaves occurred more frequently during the early hours of the day. Wet conditions facilitate the release of volatile organic compounds (VOCs) by plants (Gouinguené & Turlings, 2002; Salerno et al., 2017; Vallat et al., 2005) and thus more VOCs may be present in the early morning hours when humidity is high, making leaf preening at this time more effective. Whether time of day, rain, wetness of leaves or other factors, such as the activity of herbivorous insects (Hare, 2011), increase the release of VOCs remains to be shown. This could give greater insight into the possible function of this behaviour, but also its annual pattern. For example, if any of the above-mentioned factors triggers volatile emission, the effect on the fitness of the bird could be episodic and impact different ectoparasites at different times of the year but also lead to variation between years. On the Galápagos Islands, climatic conditions vary strongly between years (Jackson, 1993). Thus, sampling of volatile emission and preening behaviour throughout the year and over several years would be necessary for a comprehensive picture. An alternative explanation for the peak of leaf-preening behaviour in the morning is that the preening pattern follows the activity of the parasites. For instance, Amblyceran chewing lice but also the larvae of *P. downsi* have a diurnal feeding rhythm. (O’Connor et al., 2010; Stenkewitz et al., 2017).

A higher concentration of volatiles could also be the mechanism by which preening with leaves is triggered. The ability to detect VOCs through olfaction (Amo et al., 2011; Krause & Caspers, 2012; Nelson Slater & Hauber, 2017) or taste (Berkhoudt, 1992; Niknafs & Roura, 2018) has been demonstrated in several bird species, but not yet in Darwin’s finches. Testing whether Darwin’s finches are attracted to *P. galapageium* trees through olfaction or taste would be
an important step in understanding how the behaviour of preening with this species’ leaves is triggered in Darwin’s finches at a proximate level. If Darwin’s finches are able to detect the volatile compounds of *P. galapageium* leaves it seems plausible that they would also incorporate them into their nest as self-fumigation with medical plants may have beneficial effects on the health of the nestlings (reviewed in Gwinner, 2013). However, although we dismantled over 600 warbler finch and small tree finch nests since 2012 (Cimadom et al., 2019), we never found any leaves in the nest material.

In our study area, six species of Darwin’s finches are regularly present. Four of these species provided enough data for statistical analyses and they were all found to use *P. galapageium* leaves habitually in preening. Thus, it seems plausible that this behaviour is widespread among Darwin’s finches, but currently there are no data from other islands and vegetation zones. The fact that preening with leaves occurs in several Darwin’s finches suggests that this behaviour evolved before the niche specialisation of the Darwin’s finches and probably has a strong genetic component. Darwin’s finches are known for their wide range of unusual foraging techniques that are rare amongst passerines (reviewed in Tebbich et al., 2010). Tebbich et al., (2010) found high-cognitive flexibility in species that show innovative techniques but also in species that do not. In line with the “flexible stem hypothesis” (West-Eberhard, 2003), this indicates that Darwin’s finches derived from a highly flexible ancestor Tebbich et al., (2010). According to this theory, high flexibility allowed the ancestors of the Darwin’s finches to persist in a novel environment and behavioural adaptation would have been upheld by learning but later genetic accommodation (Weber & Depew, 2003) would have entrenched some or all components of this behaviour. In line with this scenario, tool use in the woodpecker finches has genetically

![Figure 4](image)

**Figure 4** Observations of preening events with leaves (A.) and observations of wet leaves (B.) plotted against time of day, separately for the non-breeding and breeding seasons.
fixed components but also components which are developed in ontogeny through individual learning (Tebbich et al., 2001). A similar scenario can be envisaged for preening with leaves, but this needs to be tested experimentally. Alternatively, this behaviour could have been discovered by one or several individuals of one species and then passed on between species by way of a cross-species transfer of information (Avarguès-Weber et al., 2013; Krebs, 1973).

Additionally, our results show that juveniles apparently only start preening with leaves in the non-breeding season, which may indicate that this behaviour needs time to mature or that Darwin’s finches need time to acquire this behaviour through social learning, as has been shown in other bird species (Slagsvold & Wiebe, 2011; Zentall, 2004). An alternative explanation is that juveniles suffer less from parasites and therefore show less leaf-preening behaviour.

In conclusion, currently we can only speculate about the function of preening with leaves of P. galapageium, but our study is the first to quantify topical application of a plant species and to report this as a predictable, habitual behaviour that varies seasonally in a closely related species group. In the non-breeding season, the frequency of this behaviour was surprisingly high (56% of all preening events), which suggests that this behaviour has adaptive value. Anointing feathers with secondary products (ants, millipedes, beetles, caterpillars, plant materials and pesticides) is taxonomically widespread, but rare, which makes it hard to evaluate the significance of this behaviour to birds’ fitness (reviewed in Bush & Clayton, 2018; reviewed in Potter, 1970). For instance, although anting has been observed in over 200 species, evidence that it reduces parasite load is scarce (reviewed in Bush & Clayton, 2018). This is probably attributable to methodological difficulties associated with measuring the effect of anointing and other forms of self-medicating (Bush & Clayton, 2018; de Roode et al., 2013).

Clayton and Wolfe (1993) established three criteria for defining self-medication behaviour: (1) the medicinal substance must be deliberately contacted by the meditator; (2) the substance must be detrimental to one or more parasites when contacted and (3) the detrimental effect on parasites leads to an increase in host fitness. Preening with P. galapageium leaves already satisfies two criteria of self-medication, as the leaves are actively applied by the birds and Cimadom et al., (2016) have demonstrated repellent qualities against potential parasites. However, we were unable to identify which parasites the birds are targeting and cannot provide evidence for parasite-reducing or fitness-enhancing effects. While we are not currently able to identify the function of anointing in Darwin’s finches, this behaviour is another example of the incredible behavioural diversity of this species group and is one of several examples in which animal behaviour has led humans to the discovery of the pharmaceutical properties of an endemic plant species (Huffman, 2002). An international research group is currently investigating short and long-term measures to mitigate the effects of P. downsii and experiments with Nerolidol, one of the main compounds of P. galapageium, are yielding the first promising results showing that it is an effective repellent of adult flies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL STATEMENT

Permission to conduct this study was granted by the Galápagos National Park Directorate (Project PC-35-19: Control of the Invasive Parasite, P. downsii and its Impact on Biodiversity). The study was observational only and did not impact the study subjects. All applicable institutional and/or national guidelines for the care and use of animals were followed.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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