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Original Article

Analysis of phoretic relation between chewing lice and hippoboscid flies of *Columba livia*

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ABSTRACT

Phoresy is a biologically mechanical phenomena where an immobile organism hitches on a other mobile organism to translocate. This behaviour is not studied very well on the field level especially between two important parasites infesting the same host. Parasite/parasite interaction is rarely studied through most biological host-parasite systems. Here, we evaluated the phoretic relation between parasitic chewing lice and hippoboscid flies (*Pseudolynchia canariensis*) on rock pigeons. A total of 69 captivated rock pigeons, *Columba livia,* were examined for the parasitic chewing lice and hippoboscid flies in Giza Zoo and two local farms near Cairo, Egypt. Results indicated that there is a positive correlation between infestation of hippoboscid flies and chewing lice. Also, the analysis of louse/louse interaction using ANOVA indicated a significant difference between the three chewing louse species which were recorded on the rock pigeons with relatively high abundance of two species, *Columbicola columbae* and *Campanulotes compar*. The analysis of hippoboscid flies' abundance and its relation with chewing lice infestation indicate a significant increase of lice intensity in case of high infestation with the fly. The level of hygiene of nest may be inversely related to level of parasite infestation. This work forms a step in the process of understanding parasite/parasite and host/parasite interactions using two parasitic species with a characteristic phoretic behaviour in nature.

1. Introduction

The order Columbiformes is a cosmopolitan group of birds found on all continents except Antarctica (Clements et al. 2019). The most widely distributed species of pigeon is the rock pigeon, *Columba livia* Gmelin 1789, which has a native range in the mountainous areas of the Old World, but today it is found on all major landmasses and many small islands. Such wide distribution has exposed rock pigeons to a wide range of environmental conditions, which may influence both host-parasite interactions and parasite-parasite interactions (Nasser et al. 2019).

Chewing lice are permanent ectoparasites that spend their entire life cycle on the host. The primary mode of dispersal of chewing lice between hosts is when two hosts come into physical contact, *e.g.* during mating or nesting seasons (Clayton and Tompkins 1994; Hillgarth 1996). Due to this close relationship with their hosts and the limited ways lice can transfer from one host to another, many chewing lice show a high degree of host specificity causing them to play a significant role in studies of host/parasite co-speciation (Johnson et al. 2003; Nasser et al. 2015a, 2020; Sweet and Johnson 2016; Sweet et al. 2018). Around 87% of bird lice are recorded only from a single host or very related bird groups (Price 1984).

By contrast, louse flies (Hippoboscidae) are capable of dispersing between hosts that are not in physical contact. The hippoboscid fly typically parasitizing rock pigeons is *Pseudolynchia canariensis* (Macquart 1839), (Soulsby 1982). Adult flies are obligatory hematophagous parasites living on the skin and feathers of their hosts and may cause irritation and be the source of disease transmission (Theodor 1975; Da Cunha Amaral et al. 2013; Hinkle and Corrigan 2013).

Inter-species relationships form at the core of biology. In fact, more than 50% of biodiversity is estimated to be parasitic. Hence, parasite/parasite and host/parasite interactions form an important subgroup of the interactions between organisms (Price 1980; Clayton et al. 2003;

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Adly et al. 2017). Interactions between parasites can be in the form of competitive exclusion (Malenke et al. 2011) or predation of one parasite species by another (Mey 2017). On the other hand, interactions between parasite species can also be beneficial for at least one parasite species; an example for that is the phoresy phenomenon by which one parasite hitch-hikes with another parasite to take a ride (**Fig. 1**), and thus may be exposed to new hosts and create a new host record (Johnson et al. 2005).

Phoresy behaviour has been well studied in the chewing louse fauna of pigeons at the laboratory level but not at the field level (Harbison et al. 2009; Bartlow et al. 2016; DiBlasi et al. 2018). In particular, two genera of ischnoceran chewing lice occurring on rock pigeons belong to different eco-morphs (Bush and Clayton 2006; Johnson et al. 2012): *Columbicola* Ewing 1929, are wing lice, and *Campanulotes* Kéler, 1939, are body lice. Previous studies have recognized that only *Columbicola* wing lice hitch-hike the hippoboscid flies as a significant transmission route (Hathaway 1943; Iannacone 1992; Clayton et al. 2004; Macchioni et al. 2005; Harbison et al. 2009; Bartlow et al. 2016).

The aim of this work is to examine the phoretic relationship between hippoboscid louse flies and chewing lice in the field by evaluating the prevalence of chewing lice on rock pigeons and compare this to the intensity of the hippoboscid flies on the same host populations at three different localities in Greater Cairo, Egypt.

2. Materials and methods

Sixty-nine captive rock pigeons were caught at three different breeding sites in Greater Cairo, Egypt. Site one was the Giza Zoo, site two a nest established by a hobbyist who trades in pigeons in central Cairo, and site three a local meat farm outside Cairo. At each site, birds kept in cages were caught by hand. Birds caught at site one, were examined in the veterinary medical unit in the Giza Zoo, whereas birds caught in the other two sites were examined on site.

Site one (Giza Zoo) represents a locality with generally good nest hygiene, as the enclosure is regularly cleaned by sanitation workers at the zoo. A total of 12 pigeons were examined at this site. Site two (a nest in central Cairo established by one of Hobbyist persons) represents a locality with moderate nest hygiene, located in an urbanized area where hobbyists regularly trade pigeons, including special breeds. A total of 21 pigeons were examined at this site. Site three (a local farm around Cairo city) represents a locality with low nest hygiene, where pigeons are raised for food purposes only. A total of 36 pigeons were examined at this site.

All pigeons were examined individually and thoroughly to establish the parasitic load. The chloroform fumigation chamber method (Clayton and Drown 2001) was used to collect ectoparasites from 12 pigeons, 21 pigeons and 36 pigeons at site one, site two and site three respectively, with fumigation followed by combing to collect all possible lice and hippoboscid flies (**Fig. 2**). Chewing lice samples were processed for slide mounting. In brief, lice were placed in 70% ethyl alcohol then dropped in Potassium hydroxide (KOH) for 15 min for clearing specimens and mounted using Puris media. The identification of chewing lice was conducted according to Tendeiro (1973 and 1974), Price et al. (2003) and Adly et al. (2019). Hippoboscid flies were identified according to Lamerton (1965). Some of the collected flies were preserved and photographed by using S-EYE YW500 camera 5mp fixed on binocular (Carl Zeiss, Standard 25 Microscope, Germany) with a special photograph' technique used according to Nasser et al. (2015b).

To avoid confusion, we here abbreviate *Columbicola* as *Co., Campanulotes* as *Ca.*, and *Colpocephalum* as *Cc.*

Fig. 1. Ventral view of hippoboscid flies *Pseudolynchia canariensis* showing phoretic association with wing lice (*Columbicola columbae*) the right one with more details with special technique according to Nasser et al. (2015b).

Fig. 2. Fumigation chamber method using for collection of ectoparasites (lice and hippoboscid flies) infesting a rock pigeon.

2.1. Statistical analysis

Means of infestation intensity of the collected ectoparasites on rock pigeons (number per pigeon) were calculated and compared by the one-way ANOVA. If such test showed significant inequality of the means, they were further analysed using pairwise comparisons by the Tukey's honestly significant difference (HSD) test. The overall prevalence (percentage of bird infested with any parasite) and prevalence of individual parasite species (percentage of the bird infested with a specific parasite species) were also calculated and tested by *F*-test. The multiple regression analysis was applied to examine the relation of the overall relative abundances (no. collected) of the hippoboscid fly (*Pseudolynchia canariensis*) with the three lice species parasitizing pigeons. The regression equation was in the form of hippoboscid fly = $a + b_1$ *Co. columbae* + b2 *Ca. compar* + b_3 *Cc. turbinatum*, where a = constant (intercept), b_1–b_3 are the slopes (regression coefficients that measure the degree of dependence of hippoboscid fly on the three lice species). The slopes were tested for deviation from zero by *t*-test. All statistical analyses were evaluated with the level of significance set to a maximum of $P < 0.01$. The PAST (Paleontological Statistics Version 2.08; 41) and the SSP (Smiths Statistical Package, Smith 2004) computerized software were used for statistical analysis.

3. Results

A total of 69 captive rock pigeons from three different sites were examined for ectoparasites. Collectively, these birds were parasitized by 2004 chewing lice specimens representing three species: the ischnoceran wing louse *Columbicola columbae* (Linnaeus 1758), the ischnoceran body louse *Campanulotes compar* (Burmeister 1838), and the amblyceran body louse *Colpocephalum turbinatum* Denny 1842. A total of 131 hippoboscid flies were also collected, all belonging to the same species, *Pseudolynchia canariensis* (Macquart 1839).

The prevalence and mean intensity of the individual ectoparasite species are given for all three sites in Table 1. All birds were positive for ectoparasites. The highest infestation rate was that of *Co. columbae* (97.10%, *P*˂0.01) followed by *Ca. compar* (92.75%), and *P. canariensis* (73.91%), with the lowest rate being that of *Cc. turbinatum* (56.52%). Means of infestation intensity were significantly different between the four parasite species (*P*˂0. 01, *F*- test).

Table 1
Infestation prevalence and intensity of ectoparasites (hippoboscid flies and lice) collected on rock pigeons, Cairo Egypt, 2019.

Ectoparasites	Prevalence (n = 69 bird)		Infestation intensity	
	No Positive	% [1]	No collected	Mean ± SD[2]
P. canariensis	51	73.91	131	3.04 ± 2.35 A
Co. columbae	67	97.10	1356	27.86 ± 12.57 B
Ca. compar	64	92.75	577	12.54 ± 6.01C
Cc. turbinatum	39	56.52	71	1.35 ± 1.80 A

1. Prevalence is statistically different (*P*˂0.01, *F*-test)
2. Means with similar letters are not significantly different (*P* ˃ 0.05, Tukey's HSD)

The prevalence for each individual site is given in Table 2 with *Co. columbae* being consistently the most prevalent parasite species (tied with *Ca. compar* in Site 2), whereas *Cc. turbinatum* was the least prevalent species in all three sites. Also, not all parasites species were found in all localities, as neither *P. canariensis* nor *Cc. turbinatum* were found at site 1. Overall, *Co. columbae* (27.86 lice/bird) and *Ca. compar* (12.54 lice/bird) were significantly more abundant (*P*˂0.01) than the other two species, which in turn were insignificantly different from each other (*P* ˃ 0.05) (Table 1).

Comparison of means of the collected hippoboscid flies and lice species per pigeon (infestation intensity) for the two sites that had the three lice species, site 2 Hobbyist nest and site 3 local farm (Table 3), revealed that both *P. canariensis* and *Co. columbae* had higher intensity in site 3 (4.47 fly/bird, *P*˂0.01; 33.72 lice/bird, *P*˂0.05, respectively) than in site 2 while both *Ca. compar* and *Cc. turbinatum* had insignificantly different intensities in the two sites (*P* ˃ 0.05). The results of multiple regression analysis (Table 4) indicated that the respective abundance of *Co. columbae*, *Ca. compar* and *Cc. turbinatum* ($b = -0.12$, 0.08 and 0.22, respectively) are directly related to the abundance of hippoboscid flies ($R^2 = 0.72$).

4. Discussion

In this study we assessed the prevalence and intensity of chewing lice parasitizing rock pigeons for the first time in Egypt. The overall prevalence rate of all parasite species combined was very high (100%); however, not all parasites were found on all hosts. This is similar to overall prevalence of parasites in other reports from the Middle East (*e.g.* Naz et al. 2010; Ahmed et al. 2017; Abdullah et al. 2018). However, Saikia et al. (2017) reported a low prevalence (39.78%) despite finding more species of chewing lice than we did. It should be noted, that of the six species of chewing lice collected by Saikia et al. (2017), five species

Table 2
Infestation by hippoboscid flies and lice on rock pigeons in the three study sites, Cairo, Egypt, 2019.

Ectoparasite species	Site 1 (n = 12)		Site 2 (n = 21)		Site 3 (n = 36)		χ^2 **
	+ve	%	+ve	%	+ve	%	
P. canariensis	0	0	18	85.71	33	91.67	219.6
Co. columbae	10	83.33	21	100	36	100	36.04
Ca. compar	9	75.00	21	100	34	94.44	36.77
Cc. turbinatum	0	0	16	76.19	23	63.89	134.14
χ^2 **		262.50		47.80		70.49	

** $P < 0.01$.
Site 1 Giza Zoo; Site 2 Hobby farm in central Cairo; Site 3 meat farm outside Cairo.

Table 3
Comparison of the collected hippoboscid flies and lice species per pigeon for site 2 (Hobby farm, n = 21 pigeons) and site 3 (meat farm, n = 36 pigeons).

	Total Collected		Mean ± SD*		$F_{1,55}$
Ectoparasite species	Site 2	Site 3	Site 2	Site 3	
P. canariensis	49	82	2.33 ± 1.46	4.47 ± 1.98	**13.58****
Co. columbae	571	647	27.19 ± 10.37	33.72 ± 10.06	5.47*
Ca. compar	249	247	13.24 ± 3.95	14.06 ± 6.08	0.30 ns
Cc. turbinatum	27	44	1.29 ± 1.19	1.83 ± 1.14	1.15 ns

* $P<0.05$; ** $P<0.01$; ns: not significant, $P>0.05$.

Table 4
The *b* (slope) and R^2 (coefficient of determination) of regression analysis for the relation of relative abundance of the three lice species with that of hippoboscid fly (*P. canariensis*).

Lice species	$b \pm SE$	R^2
Co. columbae	0.12 ± 0.01**	0.72
Ca. compar	0.08 ± 0.03 **	
Cc. turbinatum	0.22 ± 0.09 **	

** $P<0.01$, *t*-test.

have been misidentified to genus level, and at least one represents a straggler from a non-pigeon host (their "*Lipeurus caponis*" = a *Degeeriella* species; their photos of menoponids are of insufficient quality to identify these species to genus level).

Only four species of parasites were found to infest rock pigeons at the field sites in the study presented here. *Columbicola columbae* is known to be widely distributed across the range of captive pigeons, including the introduced range (Adams et al. 2005), and has previously been recorded from the Middle East (Inci et al. 2010; Naz et al. 2010; Dik and Halajian 2013; Ahmed et al. 2017; Abdullah et al. 2018). *Campanulotes compar* has been reported from Iran (Dik and Halajian 2013), Iraq (Abdullah et al. 2018), Pakistan (Naz et al. 2010) and Turkey (Inci et al. 2010). Most of these studies have also reported *Cc. turbinatum*, but this was not obtained by Abdullah et al. (2018) in Iraq. Similarly, Ahmed et al. (2017) did not obtain *Ca. compar* from Pakistan. Moreover, the louse species *Hohorstiella lata* (Piaget, 1880) and *Columbicola tschulyschman* Eichler, 1942, have been reported from the Middle East (Naz et al. 2010; Dik and Halajian 2013; Abdullah et al. 2018) but were not obtained in the present study. More research is needed to establish if these species are absent in Egypt, or if they are just absent in the sites sampled for this study.

The prevalence of the three lice species indicated that out of 69 pigeons examined for ectoparasites, *Co. columbae* had the highest prevalence. This is in agreement with Naz et al. (2010), who also found *Co. columbae* to be the most frequent parasite of captive pigeons. *Columbicola columbae* as the most common Mallophagan parasite of pigeons was also previously reported by Harlin (1994); Musa et al. (2011); Dik and Halajian (2013) and Ahmed et al. (2017).

Based on means of infestation intensity (ectoparasite/bird), *Co. columbae* (27.86) and *Ca. compar* (12.54) were significantly more abundant ($P<0.01$) than the other two species; the infestation intensity of *P. canariensis* and *Cc. turbinatum* was not significantly different ($P>0.05$). The reported ranges of prevalence and mean intensity by several authors are 59–98.6% and 17.9–179.3 for *Co. columbae* and 26–91.8% and 5.4–153.6 for *Ca. compar* (summarized by Naz et al. (2010).

The prevalence of all four parasites species was higher in the two field sites where general hygiene was lower (Sites 2–3); *P. canariensis* and *Cc. turbinatum* were absent in site 1 where general hygiene was higher. Comparing the two sites where *P. canariensis* was present, intensity of both *P. canariensis* and *Co. columbae* was higher in site 3, which had lower general hygiene than site 2. No significant difference in intensity was observed between these two sites for *Ca. compar* and *Cc. turbinatum*. More sites of different hygiene level need to be examined to establish whether this pattern holds, and to examine what causal relationships may exist between general hygiene level and the infestation intensity of pigeon parasites.

The regression model with the abundance of the hippoboscid fly as an explanatory (predictor, independent) variable and the abundances of the three lice species as dependent (criterion) variables showed direct relation and that 72% of the total variance in hippoboscid fly abundance was accounted for. This may be explained by the phoresy of *Columbicola* on *Pseudolychia* which may homogenize infestation of *Co. columbae* throughout the host population. The remaining 28% of the variance may be attributed to factors related to the host (age, sex and breed) or to the external environment (number of birds in the nest, sanitation, *etc.*) (Nadeem et al. 2007). These kinds of relations draw a very impressive understandable picture about parasitism relationships. Exploration of such biological relations and phenomena is critical to further our understanding of fauna. Using new trends like GIS and molecular investigations could be useful in furthering such understanding (Hosni et al. 2020; Kamal et al., 2020; Okely et al., 2020).

Ethical statement

All captured birds were examined for chewing lice and other parasites following the regulation of Research Ethics Committee of faculty of science, Ain Shams University. Then all examined birds were released at the capturing location without any harm.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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