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ORIGINAL ARTICLE

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Is the citrus-like plumage odorant of crested auklets (*Aethia cristatella*) a defense against lice?

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Abstract Plumage odors may function as chemical defenses against ectoparasites in birds. We tested this hypothesis for crested auklets (*Aethia cristatella*), a species of colonial seabird that emits a very strong citrus-like odor from its plumage. This odorant contains known chemical repellents. We evaluated evidence for chemical defense in this species using two approaches. First, we exposed pigeon lice to the volatiles emitted by freshly plucked plumage and by whole specimens. Louse survivorship was compared between these treatments and two controls. Second, we compared louse abundance on crested auklets versus a closely related congener that nests in close association. Louse survivorship did not differ between crested auklet treatments and controls. Comparison of ectoparasite loads showed that crested auklets had significantly higher louse abundance than least auklets (*A. pusilla*), even after controlling for body size. Our results failed to support two expectations for chemical defense. Presence of the crested auklet plumage odorant in our experiments did not reduce louse life span. Presence of the aldehyde odorant in nature did not reduce louse abundance on crested auklets. Hence we conclude that the aldehyde odorant is not immediately lethal to lice at natural concentrations in plumage.

Keywords Avian plumage odors · Chemical defense · Chewing lice · Crested auklet · Ectoparasite load

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Introduction

Strong odors may be indicative of chemical defense in birds (Dumbacher et al. 1992; Dumbacher and Pruett-Jones 1996; Weldon and Rappole 1997), similar to the way in which strong odors are associated with defensive secretions among insects (Eisner 1970). Crested auklets (*Aethia cristatella*) are pigeon-sized colonial seabirds from the North Pacific that emit a citrus-like odor (Humphrey 1958). The chemical constituents of the odorant suggest a defensive benefit. Short-chained aldehydes comprise the auklet odorant, and two of the major constituents are potent invertebrate repellents, suggesting that the citrus-like odorant may repel ectoparasites (Douglas et al. 2001). Experiments have shown that synthetic analogues of the crested auklet odorant kill lice and repel ticks in very small quantities (Douglas et al. 2004). This would not be without precedent. Some materials applied topically or sequestered in bird feathers are known to kill parasites. Clayton and Vernon (1993) showed that lime vapor kills lice, and Dumbacher (1999) showed that the toxic feathers of pitohui birds in New Guinea also kill lice. Chemical scents in vertebrates may also be acquired through natural or sexual selection for reasons unrelated to chemical defense (Darwin 1871; Blaustein 1981; Gorman and Trowbridge 1989). This might be true for crested auklets. Conspicuous sexual and social behaviors suggest that the auklet odorant is important in courtship (Hunter and Jones 1999; Douglas et al. 2001; Hagelin et al. 2003; Jones et al. 2004). Thus, avian plumage odors like the crested auklet odorant might be a form of chemical adornment and not a means of chemical defense. We assessed evidence for chemical defense in crested auklets using two approaches. First, we tested the hypothesis that lice from rock pigeons (*Columba livia*) would not survive exposure to crested auklet plumage as well as to controls. Second, we tested the hypothesis that ectoparasite loads of crested auklets would differ from those of least auklets (*Aethia pusilla*). Our first approach utilized lice from rock pigeons in survival experiments similar to those of Clayton and Vernon (1993) and Dumbacher (1999). The advantage of this experimental design is that pigeon lice can be obtained in large numbers and are known to be killed both by lime vapor (within 9 h, Clayton and Vernon 1993) and pitohui feathers (within 35 h; Dumbacher 1999). We used two species of lice that are host specific parasites of rock pigeons. The two species, *Columbicola columbae* and *Campanulotes compar*, are permanent parasites that are restricted to the body of the host by appendages specialized for locomotion on feathers (Clayton 1991). The two species are ''ecological replicates'' that complete their entire life cycle on the body of the host, where they feed on feathers and dermal debris (Clayton and Johnson 2003). Transmission among hosts occurs mainly during physical contact between the feathers of different individual birds, such as that between mated individuals or between parents and their offspring in the nest (Marshall 1981). In our second approach, we determined the ectoparasite loads of individual crested and least auklets caught in the same colony. Least auklets live in the same mixed breeding colonies with crested auklets, but do not have a noticeable plumage odor. Least auklets are closely related to crested auklets (Friesen et al. 1996). At our research site, crested and least auklets socialize on some of the same landing rocks, and nest near each other in rock crevices. In this second approach, we performed body washing of individual specimens to assess diversity and abundance of lice.

Methods

Survival experiments

We tested the hypothesis that the crested auklet's plumage odor kills ectoparasitic lice (Phthiraptera: Insecta). We conducted two experiments on St. Lawrence Island, Alaska, during August 2001. In experiment I, we filled petri dishes with freshly plucked feathers from the nape and upper back. In experiment II, we placed whole specimens in beakers. Each experiment had three treatments—crested auklet, least auklet, and rock pigeon. Experiment I used three replicates for each treatment. Experiment II used three replicates for crested auklets and least auklets but only two replicates for rock pigeons. Crested auklet treatments continued to emit the characteristic citrus-like odor throughout the trials in both experiments, but the other treatments did not emit a noticeable odor. Experiment I had the advantage of concentrating the feathers and their associated volatiles within a small volume. Experiment II had the advantage of using whole specimens that probably retained the aldehyde odorant for a longer period and emitted more of the chemical. However, in experiment II the volatile chemicals would have diffused in a larger air volume. In both experiments the lice were placed on a piece of porous filter paper suspended just above the feathers, preventing direct contact by lice, similar to the design used by Clayton and Vernon (1993). This approach allowed us to keep track of individual lice and controlled for any differences in survival owing to differences in the substrate itself, rather than odor, per se.

Survival experiments: petri dishes

Experiment I Feathers were plucked from the nape and upper back of a recently killed bird (within 8 h of death). Sufficient feathers were plucked to fill the base of a petri dish. The feathers were covered in the base of the dish with a piece of porous filter paper. Six *Columbicola columbae* and six *Campanulotes bidentatus* were placed on top of filter paper covering the feathers, and the petri dish was covered with a lid. In two replicates there was one fewer louse due to shortage. The two species of lice were pooled as a single category. Survival of the lice was determined after a period of 30 h by examining each louse closely under a dissection microscope. A few lice managed to escape from the dishes during the 30-h trial. The rate of escape was independent of treatment, so those lice that escaped were reported in our results but excluded from the statistical analysis of survival.

Survival experiments: beakers

Experiment II This experiment also had three treatments with three beakers per treatment for crested and least auklets, and two beakers for the rock pigeon treatment (because we had only two pigeons). A recently killed bird of the relevant species was placed in each beaker at the start of the experiment. Ten lice of each species were placed on top of filter paper suspended in the mouth of the beaker, which was then sealed with aluminum foil. In one replicate there was one extra louse. Again, the two species of lice were pooled as a single category. Survival of the lice was determined after a period of 30 h using the same criteria as for the petri dish experiment.

Comparison of natural louse loads

We compared the relative loads of 21 crested and 25 least auklets sacrificed for other studies. These specimens were obtained from the same nesting area in the Myauk colony on St. Lawrence Island, Alaska, on 4–5 August 2001. Specimens were immediately placed inside individual paper bags and sealed in ziploc plastic bags. The specimens were shipped frozen to the Clayton laboratory at the University of Utah where louse loads were quantified using the body washing method of Clayton and Drown (2001). Lice were tabulated for each specimen and identified to species (*Quadraceps aethereus*, *Saemundssonia wumisuzume*, *S. boschi*, *Austromenopon nigropleurum*). The same species of *Quadraceps* and *Austromenopon* occur on crested and least auklets. *S. boschi* (a new species of avian louse) was found only on least auklets, and *S. wumisuzume* occurs on crested auklets but not least auklets (Price et al. 2003). Data on ectoparasite loads were adjusted for body mass because crested auklets (mass = 252 g, SE = 3.6, $n = 21$) are 300% larger than least auklets (mass = 84 g, SE = 1.2, $n = 17$). The abundance of lice on least auklets was multiplied by a numerical coefficient (2.17) derived from Clayton and Walther (2001, Fig. 2b), who demonstrated a predictable relationship between avian ectoparasite load and body size of the host. The corrected data for least auklets were compared to ectoparasite loads of crested auklets with a Mann-Whitney *U* test.

Results

Survival experiments: petri dishes

Fewer lice escaped from petri dishes with crested auklet plumage (17.6%) than those with least auklet (22.2%) and rock pigeon plumage (33.3%), but this difference was not significant ($\chi^2 = 2.5$, $df = 2$, $P = 0.29$, Table 1). Louse survival did not differ between treatments either (Table 1): 42.9% of lice over crested auklet feathers survived for 30 h, compared to 39.3% of lice over least auklet feathers, and 41.7% of lice over the (control) rock pigeon feathers ($\chi^2 = 0.08$, $df = 2$, $P = 0.96$).

Survival experiments: beakers

More lice escaped from the filter paper in the least auklet treatment (30%) than those lice in the crested auklet (9.8%) or rock pigeon (10%) treatments ($\chi^2 = 10.6$, $df = 2$, $P = 0.005$, Table 1). It was not possible to positively determine the fate of escaped lice, and we do not know why more lice escaped from some treatments. The observation demonstrates that lice were capable of climbing and escaping from the filter paper. Louse survival among the three treatments in this experiment was even more similar than in the previous experiment: 47.3% of lice over crested auklets survived for 30 h, compared to 47.6% of lice over least auklets, and 44.4% of lice over rock pigeons. Again, the differences were not statistically significant ($\chi^2 = 0.09$, $df = 2$, $P = 0.95$). Survival experiments were ended after 30 h because there were no apparent trends in mortality, some lice had escaped, and the potency of the auklet feathers had likely diminished over time.

Comparison of ectoparasite load

Crested auklets had higher louse loads than least auklets (Mann-Whitney: $P < 0.001$, two-tailed). Two species of louse were more abundant on crested auklets than least auklets—*Q. aethereus* (Mann-Whitney $P < 0.001$, two-tailed), *Saemundssonia* sp. (M-W, $P < 0.001$, two-tailed). However, abundances of *A. nigropleurum* did not differ between crested and least auklets (Mann-Whitney $P = 1.0$, two-tailed). Even after adjusting for differences in host body size, crested auklets still had significantly more lice than least auklets (Mann-Whitney $0.009 < P < 0.01$, two-tailed). In this comparison, the difference was principally due to a higher abundance of *Quadraceps aethereus* (Mann-Whitney $0.005 < P < 0.006$, two-tailed) on crested auklets. The abundances of other louse species did not differ between crested and least auklets (*Saemundssonia* sp., $0.09 < P < 0.1$; *A. nigropleurum* $1.0 < P < 1.1$, Mann-Whitney two-tailed).

Discussion

There was no support for the predicted differences in louse lice span or louse abundance. Exposure of lice to volatiles from crested auklet plumage did not reduce life span for pigeon lice. Survivorship of lice was similar regardless of whether the lice were suspended over plumage of least auklets, crested auklets, or rock pigeons. Crested auklets also had higher abundances of lice than least auklets, even after adjusting for host body size. This demonstrates that the presence of the aldehyde odorant in nature does not reduce louse abundance relative to a conspecific dwelling in close association at the same colony. Observations collected

Table 1 Results of louse survival experiments on feathers and specimens of crested auklets (*Aethia cristatella*), least auklets (*A. pusilla*) and rock pigeons (*Columba livia*)

Treatment	Stayed	Escaped	Totals		Alive	Dead	Totals	
Experiment 1: petri dishes					Survival results for those lice that stayed			
Crested auklet feathers	28	6 (17.6%)	34	$\chi^2 = 2.5$	12 (42.9%)	16 (57.1%)	28	$\chi^2 = 0.08$
Least auklet feathers	28	8 (22.2%)	36	$df = 2$	11 (39.3%)	17 (60.7%)	28	$df = 2$
Rock pigeon feathers	24	12 (33.3%)	36	$P = 0.29$	10 (41.7%)	14 (58.3%)	24	$P = 0.96$
Experiment 2: beakers					Survival results for those lice that stayed			
Crested auklet specimens	55	6 (9.8%)	61	$\chi^2 = 10.6$	26 (47.3%)	29 (52.7%)	55	$\chi^2 = 0.09$
Least auklet specimens	42	18 (30.0%)	60	$df = 2$	20 (47.6%)	22 (52.4%)	42	$df = 2$
Rock pigeon specimens	36	4 (10.0%)	40	$P = 0.005$	16 (44.4%)	20 (55.6%)	36	$P = 0.95$

during the experiment suggest that lice may not have been repelled by the crested auklet treatment. We noted that fewer lice escaped from the crested auklet treatments than the controls. This might have happened because subtle differences in the placement of the filter paper allowed more lice to escape from some beakers and dishes. Alternatively, fewer escapes might be indicative of impairment of lice by the crested auklet odorant, thus reducing their mobility.

Our results suggest that the crested auklet odorant does not function as a chemical defense. However, there are caveats. Crested auklets had higher abundances of lice than least auklets. This might be attributable to differences in sociality. Crested auklets are highly gregarious, more so than least auklets (Gaston and Jones 1998). This brings crested auklets into close contact with each other on a frequent basis, and this contact affords increased opportunities for louse transmission. Furthermore, our experiments may not reproduce the same conditions as live birds. First, lice in our treatments did not come in contact with plumage. Second, the aldehydes are transient in plumage due to their volatile and reactive qualities. Trials with synthetic analogues of the crested auklet odorant show that the efficacy of the odorant as a repellent begins to wane 1 h after application (Douglas et al. 2004). This is similar to what has been shown for commercial repellents such as DEET (Dautel et al. 1999). Interpretation becomes further complicated when we consider that the crested auklet odorant may require a longer period to act than the duration over which the experimental data have been collected. Pitohui feathers of lower toxicity required longer periods to inflict mortality (>100 h for some louse species; Dumbacher 1999). Synthetic analogues of the crested auklet odorant are strongly repellent to ticks and exposure to very small quantities kills lice instantly (Douglas et al. 2004). However, these effects are dose dependent, and the transience of the odorant may have resulted in reduced dose over the period of our trials. Constituents of the crested auklet odorant are lethal to lice (Douglas et al. 2004). However, our results suggest that the crested auklet odorant is not immediately lethal to lice at naturally occurring concentrations. Instead the odorant may have sublethal effects such as repellence, impairment, or delayed development.

Zusammenfassung

Wirkt der zitronen-ähnliche Gefiederduft des Schopfalks (Aethia cristatella) gegen Federläuse?

Wir prüften die Frage, ob Gefiederdüfte als chemische Abwehr gegenüber Ektoparasiten wirken können, am Schopfalk, einem koloniebrütenden Seevogel, von dem bekannt ist, dass er stark zitronen-ähnlich duftet. Diese Duftstoffe enthalten bekannte chemische Abwehrstoffe. Zunächst testen wir die Reaktion von Taubenläusen gegenüber diesen flüchtigen Stoffen entweder von frisch gezognen Federn oder des ganzen Vogels. Die Überlebensrate der Läuse wurde zwischen diesen Ansätzen und gegenüber zwei Kontrollen vergleichen. Weiterhin verglichen wir die Befallsdichte mit Läusen an Schopfalken mit der in der gleichen Kolonie brütender Zwergalke. Die Überlebensrate der Läuse unterschied sich nicht zwischen den verschieden Behandlungen und den Kontrollen. Die Schopfalke hatten einen signifikant höheren Läusebefall als Zwergalke, auch wenn wir die unterschiedliche Körpergröße berücksichtigten. Damit konnten wir die Hypothese einer möglichen chemischen Ektoparasitenabwehr nicht bestätigen. Da der Duftstoff weder die Überlebensrate der Läuse noch die Befallsdichte beeinflusste, schließen wir, dass das im Duftstoff enthaltene Aldehyd in natürlich vorkommender Konzentration nicht unmittelbar lethal für Federläuse ist.

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