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Colonial seabird's paralytic perfume slows lice down: An opportunity for parasite-mediated selection?

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ABSTRACT

Selection for chemical signals in birds could be influenced by parasitism as has been previously suggested for visual and acoustic displays. Crested auklets (*Aethia cristatella*), colonial seabirds from Alaska and Siberia, offer an example of how this might occur. Crested auklets secrete lipids in plumage, possibly as an indicator of status and attractiveness. Prominent among these secretions are aldehydes, which are noticeable as a pungent citrus-like odour. Octanal and hexanal, the most abundant aldehydes in the plumage of crested auklets, are potent invertebrate repellents, reported from the chemical defenses of heteropteran insects. These aldehydes occur at high concentrations within specialized secretory structures. Experiments presented here show that these compounds can paralyse lice. Lice obtained from auklets were paralysed or killed within seconds after exposure to volatiles from nicks in the integument of a crested auklet. Chemical analysis demonstrated the presence of aldehydes in the area of integument used for this experiment. Lice exposed to control tissues in the same manner were not affected. A synthetic blend of crested auklet odourant constituents caused a sequence of impaired behaviours in auklet lice comparable to the effects of neuroactive insecticides. The time until onset of effects was dependent on dose, suggesting that the rate of molecular diffusion into louse spiracles was the explanatory factor. Impairment was evident even at very low concentrations that can occur in crested auklet plumage during winter. The same aqueous emulsions were present in both experimental and control treatments but lice in controls experiments were not affected. Crested auklets inhabit crowded social neighbourhoods with larger social groups, closer interindividual spacing and higher rates of contact than sympatric least auklets (*Aethia pusilla*). This could help to explain why crested auklets can have higher louse abundances. Lice are spread through direct contact between hosts and louse-infected mates could transmit lice to offspring. Large differences in the louse loads on crested auklet fledglings suggest differences in the parental transmission of lice to offspring.

1. Introduction

Invertebrates may use the same chemical compounds for communication and defense, but this is virtually unknown in birds. Heteropteran insects offer numerous examples of such dual functions (Blum, 1981; Aldrich, 1988). The crested auklet (*Aethia cristatella*), a colonial seabird from Alaska and Siberia, secretes chemical repellents in plumage that are also known from the chemical defenses of heteropteran insects. Octanal and hexanal, the two most abundant aldehydes in the plumage of crested auklets (Douglas et al., 2001, 2004; Douglas, 2008a) are potent invertebrate repellents reported from the chemical defenses of heteropteran insects (Waterhouse and Gilby, 1964; Blum, 1981; Aldrich, 1988; Eliyahu et al., 2012). Crested auklets are attracted to the aldehyde constituents of their citrus-like odourant (Hagelin et al., 2003; Douglas, 2008a). These compounds may be important in communication (Douglas et al., 2001, 2004; Hagelin et al., 2003; Jones et al., 2004; Douglas, 2008a; Hagelin and Jones, 2007; Hagelin, 2007). The specific composition of aldehydes could be important for signaling, particularly since two dodecenals appear to occur in a consistent 1:1 ratio (Douglas et al., 2004; Douglas, H.D., 2006. Odours and ornaments in crested auklets (*Aethia cristatella*): signals of mate quality? PhD Dissertation, University of Alaska Fairbanks, Alaska; Douglas, 2008a). Aldehydes could also help to volatilize other compounds in plumage, increasing the active space of those signals.

It has been suggested that visual and acoustic displays can serve as indicators of intrinsic quality in the signaler, if parasite load affects signal expression (Hamilton and Zuk, 1982). For example, high parasite load in male house finches (*Carpodacus mexicanus*) is correlated with reduced development of plumage coloration, a

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sexually selected trait (Thompson et al., 1997). Both endoparasitic and ectoparasitic infections may affect physiological condition and impair expression of secondary sexual traits in male house finches (Thompson et al., 1997). Parasite load can also affect song performance in birds (Garamszegi, 2005).

Many species of birds are reported to emit odors (Weldon and Rappole, 1997), and it is possible that chemical signals could function as indicators of condition. For example, the crested auklet's aldehyde odourant may be derived from fatty acid metabolism (Douglas et al., 2001, 2004). Individual aldehydes occur in specialized wick feathers with their complementary fatty acid methyl ester and carboxylic acid (Douglas, PhD dissertation (cited earlier), 2008a). Fatty acids are valuable fuels and their allocation is tightly regulated (Nelson and Cox, 2004). Chemical signals are different from visual and acoustic signals in that the signal itself could have direct effects on parasites. For example, constituents of the crested auklet odourant repel or impair ticks, lice and mosquitoes (Douglas et al., 2004, 2005a,b). Gregarious social behaviour could predispose crested auklets to higher prevalence and intensity of louse infestations (Douglas et al., 2005a), and this could be a selection pressure favouring the crested auklet's aldehyde odourant. This would be consistent with what has been noted for colourful visual traits. Among birds the species that are exposed to higher levels of parasitism have more brightly coloured males (Hamilton and Poulin, 1997; Poulin and Forbes, 2012). Evolution of the crested auklet odourant could have involved mutual reinforcement by sexual and natural selection. Increased secretion of volatiles could elevate the status of the signaler while also helping to control parasites. The crested auklet's aldehyde odourant could function as a signal of mate quality (Douglas et al., 2001), because it could signal an individual's resistance against ectoparasites, its capacity to allocate endogenous resources to signal production and its attractiveness (Douglas et al., 2001, 2004, 2005b; Douglas, PhD dissertation (cited earlier), 2006, 2008a).

Aldehydes could mediate the host–parasite relationship in crested auklets and it is important to understand how the compounds are perfused in plumage. Wick feathers contain a solution with highly concentrated aldehydes (Douglas, PhD dissertation (cited earlier), 2008a). During alloanointing crested auklets appear to apply pressure on the interscapular area of a display partner with their bill, and this could forcibly expel aldehydes through translucent wick-like hairs (Douglas, PhD dissertation (cited earlier), 2008a). When aldehydes are released from secretory tissues they can volatilize rapidly, similar to the emissions of some heteropteran insects (Blum, 1981; Aldrich, 1988; Eliyahu et al., 2012). Crested auklets have dense layers of downy feathers and contour feathers; the finely branched structure of interlocking feather barbs traps air (Hanson, 2011). Volatile aldehydes can diffuse in this matrix, adsorb to surfaces of feathers, and be absorbed into oil emulsions on feathers. Hexanal and octanal partition strongly into liquid alkane emulsions (Coupland, 2006), and this phenomenon can be generalised for oils (Rabe et al., 2003a). The affinity of volatile aldehydes for lipid emulsions varies with physical state, molarity, chain length, the sequence of fatty acids and the degree of saturation (Maier, 1975; Le Thanh et al., 1992; Coupland, 2006; Rabe et al., 2003a,b). Hexanal and octanal can be rapidly excluded from emulsions (Coupland, 2006). Some studies have attempted to use plucked feathers to assay effects of plumage odors on lice (Douglas et al., 2005a; Hagelin, 2007). These authors did not report the aldehyde concentrations that were used in treatments, although they did note a citrus-like odour. A citrus-like scent might be attributable to putative oxidation products (e.g., octanoic acid). These early efforts were patterned after methods better suited for relatively inert compounds (e.g., homobatrachotoxin, Dumbacher, 1999), and a problem with this approach is that the feathers may be only a temporary substrate for volatiles. Plucking of feathers could disrupt the molecular matrix in plumage causing aldehydes to be dispersed. As a result the content of volatiles of plucked feathers may not be the same as the plumage in the integument of a living bird.

In order to understand the interaction between host and ectoparasites, it is useful to study dose response. Observations from a previous study (Douglas et al., 2004), suggested that the crested auklet odourant could have effects similar to the neuroactive substances that some terrestrial birds use for self-anointment (e.g., pyrethrum in marigolds, D-limonene in citrus, Ehrlich et al., 1986; Clayton and Vernon, 1993; Clayton et al., 2010). The hypothesis that the crested auklet odourant does not impair lice was tested. Lice were exposed to concentrations of synthetic aldehydes similar to those that can occur in the plumage of free-living crested auklets. Lice were also exposed to volatiles from the secretory tissues of a crested auklet by making a nick in the integument with the tip of a scalpel. The rationale for this experiment is that aldehydes present in secretory tissues can be released to plumage rapidly during alloanointing. While it is difficult to measure this empirically, some inferences can be made. The maximum flux of octanal that has been measured for a crested auklet at rest is 19.9 μl/50 min (Douglas, 2006). The octanal flux that can occur during alloanointing is probably greater than this. Concentrations of octanal can be 590 μg/g in plumage and up to 9.5 μg/cm^2 on accessory bill plates for crested auklets that have recently been involved in alloanointing (Douglas, PhD dissertation (cited earlier), 2008a). Furthermore, nearly pure octanal has been collected in the headspace above the napes of frozen crested auklet specimens as they thawed (Douglas et al., 2001).

Avian lice are transmitted socially (Marshall, 1981), and they could spread in a manner similar to infectious diseases. Sociality increases the opportunities for transmission of infectious diseases, because the probability of an individual becoming infected depends upon the number of agents it interacts with (McCallum et al., 2001; Ferrari et al., 2011). I adopt the term "social neighbourhood" after Ferrari et al. (2011) and others to characterise frequency- and density-dependent factors in social networks. Direct contact among birds provides lice with opportunities to move between hosts. Although it is not possible to observe the transmission of lice between birds, the frequency and number of different social contacts could be an explanatory factor for louse transmission rates. Highly connected social networks could allow lice to spread rapidly from reservoirs within the host population when conditions are favourable. Crested auklets are reported to be the most gregarious of the *Aethia* auklets (Gaston and Jones, 1998), but there are no published quantitative data to compare social neighbourhoods of crested auklet and least auklets (*Aethia pusilla*). The most observable social interactions for these species occurs on the surface of colonies during daily activity periods. This is where the largest number of birds interacts within the smallest area. These interactions may represent the greatest potential for louse transmission. Crested and least auklets attend their colonies en masse to swamp predators. They alight on landing rocks and disperse to socializing rocks and nest crevices. Generally the surface of the colony is rock talus or volcanic lava. I compared social group size, interindividual spacing, and rates of contact for crested auklets and least auklets. By social group I mean conspecifics that have the potential to come into contact due to their proximity within the colony. I tested the hypothesis that there would be no difference in these parameters with respect to species. Crested and least auklets appear to be habitual in their patterns of colony attendance. Therefore, it is reasonable to speculate that lice may spread through social networks within colonies, as has been generally proposed for wildlife populations (Ferrari et al., 2011).

Crested auklets that secrete greater amounts of aldehydes may have greater resistance against ectoparasites and transmit fewer ectoparasites to mates and offspring. Auklet chicks have few defenses against lice. High louse abundances could pose energetic costs for juveniles due to the additional time and energy needed for self-maintenance (Cotgreave and Clayton, 1994). Lice can cause loss of feather insulation, which in turn can increase the heat flux from the bird's body to the environment (Booth et al., 1993). Birds compensate for increased thermal conductance by increasing metabolic rate or suffer a reduction in body temperature (Booth et al., 1993). Thermal conductance refers to a coefficient of heat transfer (p. 374, Ellis and Gabrielsen, 2002). Loss of feather insulation could reduce the thermoneutral zone, the temperature range over which metabolism can remain basal (Stahel and Nicol, 1982; Ellis and Gabrielsen, 2002). The consequences for increased thermal conductance can be more costly for marine birds because water has a thermal conductivity 25 times greater than air (Stahel and Nicol, 1982). Louse infestations could impose fitness costs for juvenile crested auklets and this could be a basis for parasite-mediated selection. I tested the hypothesis that there would be low variance in the intensity of louse infestations on crested auklet fledglings. Departures from this pattern represent variance in the prevalence and intensity of parasitism, which may covary with parasite-mediated selection (Clayton et al., 1992).

2. Materials and methods

2.1. Bioassays

Experiments to simulate the effects of the crested auklet odourant on avian feather-feeding lice (Phthiraptera: Ischnocera) were conducted at Buldir I., Alaska in July and August 2003 and at St. Lawrence I., Alaska in June 2005 with auklet lice of the genera *Austromenopon*, *Quadraceps* and *Saemundssonia*. In experiment 1, lice were exposed to volatiles from nicks made in the integument of a male crested auklet with a scalpel. In experiments 2 and 3, auklet lice were exposed to volatiles from synthetic auklet odourants for controlled comparisons of dose effects. Lice from experiments 1–3 were identified and accessioned in the laboratory of Dr. Dale Clayton at the University of Utah, USA. Experiment 4 tested the effects of very low doses of synthetic auklet odourants on ectoparasitic pigeon lice (*Columbicola columbae* and *Campanulotes compar*). This experiment was helpful for comparative purposes. Experiment 4 was conducted at the University of Utah in August 2005 in the laboratory of Dr. Dale Clayton. These experiments approximated the range of effects that occur in nature from exposure to volatiles alone. Lice were placed on feathers that lacked aldehydes. Crested auklet feathers used in experiments 1–3 were collected in 2002 at Big Koniuji I., Alaska. The feathers were heated at low temperature in an oven to volatilize lipids. Feathers used in experiment 4 were collected from captive rock doves (*Columba livia*); the birds were also the hosts for the pigeon lice used in experiment 4. In all experiments, lice were handled with a fine hair camel brush, and a headset with 2× and 4× magnifying lenses was used during handling and transfer.

In experiment 1, lice were briefly exposed to volatiles from shallow nicks in the integument of a freshly killed breeding adult male crested auklet. This specimen was obtained on July 23, 2003 at Buldir I. and the presence of a brood patch confirmed its breeding status. The experiment commenced immediately because cells can begin to lyse soon after the death of an organism. The specimen was placed under a dissecting microscope on a boom arm (Bausch & Lomb Stereozoom 7), and a shallow nick was made in the integument with the tip of a scalpel. A louse was placed on a toothpick and held in close proximity over the nick for 12 s. The louse was transferred to a feather and placed in a vial. This procedure was repeated for each louse exposure. The rationale for this experiment was that aldehydes are highly volatile and they could have immediate effects upon lice when perfused in plumage. This experiment tests whether there were sufficient concentrations of aldehydes in the integument to affect lice. Nicks for experimental treatments were made in the interscapular region and the nicks for control treatments were made on the flank. Aldehydes of the crested auklet odourant have not been found on the flanks (unpublished data). Four lice obtained from adult crested auklets and three lice obtained from adult least auklets were used for experimental treatments. Two lice obtained from adult crested auklets and two lice obtained from adult least auklets were used for control treatments. These were all the lice that could be obtained in time for this experiment. Observations of lice in vials were made with the dissecting microscope at regular intervals until 95.5 h elapsed time.

In experiments 2–4, lice were placed on feathers and inserted into an inverted vial, and a cotton ball containing the treatment was placed inside the vial cap at the base of the feather. Johnson & Johnson sterile cotton balls were used for experiments 2–4 because this product has a consistent size and weight. Cotton was chosen because it is absorbent and has a low density similar to feathers. The cotton balls had a mass of 0.2963 g (S.D. = 0.025, $n = 10$, scaled with a Mettler Toledo AL204 balance). The apparent density of the cotton balls based upon uncompressed volume was relatively low (0.009 g/cm^3) compared with density of feathers (0.8 g/cm^3 for chicken feathers, Reddy and Yang, 2007). The value for the relative density (specific gravity) of cotton fibre is higher (1.52, Warner, 1995, p. 40) than values cited for feathers (0.8 to 1.2 for chicken feathers, Rock et al., 2005). Experiment 2 was conducted at ambient environmental temperature (7–13° C); experiments 3 and 4 were conducted at approximately 21 °C.

Bioassays in experiments 2–4 tested for dose-dependent responses by simulating effects of the crested auklet's aldehyde odourant on lice. Concentrations of a synthetic aldehyde blend were varied as the explanatory variable. The dependent variable was the 100% effective dose (ED), which was defined as the dose at which 100% of lice in a replicate were affected. This criterion was used because it was unambiguous for nonlethal effects. Louse behaviour was classified as follows: normal active, reduced ambulation with slowed articulation of appendages, reduced ambulation with spastic or jerky movements of appendages, non-ambulatory with spasmodic twitching, immobilised, and moribund. These categories of behaviour represent different levels of impairment and are similar to the sequence of behaviours described for neuroactive insecticides (Corbett et al., 1984). These categories are recognisable for arthropods and can be applied objectively. Experiment 2 was conducted as a binary quantal response bioassay with a serial sampling design (after Robertson and Preisler, 1992). Sample sizes were 15 lice per replicate. Initially vials were observed every few minutes until all lice were moribund and subsequently at 45 min, 8 and 18 h elapsed time. Experiment 3 tested for dose response at very low concentrations. Only six lice could be obtained after fumigating 11 specimens of breeding adult crested auklets with CO_2. These specimens had relatively high concentrations of aldehydes in their plumage (Douglas, PhD dissertation (cited earlier), 2008a), and that might help to explain why there were so few lice. Two auklet lice were added to feathers in each vial. Observations were made at regular intervals for 46 h. Experiment 4 tested for a dose response effect at very low concentrations and the 100% ED was the elapsed time at which all lice manifested reduced ambulation with spastic or jerky movements of appendages. Nine pigeon lice were added to pigeon feathers in each vial. Observations were made at regular intervals for 46 h.

Bioassays were similar to the concentrations of aldehydes that can occur in free-living crested auklets. In experiment 2 the stock solutions were 100%, 25% and 12.5% aldehydes, with resulting total aldehyde concentrations of 0.34, 0.30, 0.20 and 0.10 mg/g in cotton. This was similar to concentrations of aldehydes that can occur in crested auklet plumage during alloanointing. For example, octanal, hexanal and (*Z*)-4-decenal can attain 0.590, 0.528 and 0.393 mg/g, respectively (Douglas, 2008a). In experiments 3 and 4 the stock solutions were 1.00%, 0.50% and 0.10% aldehydes, with resulting concentrations of 0.067, 0.034 and 0.0067 μg/g in cotton. The 0.50% treatment is similar to aldehyde concentrations that can occur in wild crested auklets during winter (unpublished data). Experiment 4 had one additional treatment with a stock solution of 0.05% and a resulting concentration of 0.0034 μg/g in cotton. Treatments were dispensed with syringes or calibrated pipettes. The same quantities of control solution were dispensed into experimental and control treatments. This control solution for experiments 2–4 was one part 95% ethanol mixed with nine parts aqueous glycerin solution. The aqueous glycerin solution was three parts distilled H_2O to one part glycerin. Aldehydes are hydrophobic, and the addition of ethanol and glycerin was necessary to make aldehydes miscible in an aqueous solution. The control solution did not affect lice. However, ethanol can affect lice if used in higher concentrations or larger doses. Experimental treatments were emulsified in the control solution by vigorous mixing. The crested auklet odourant treatment was formulated as follows: 9.4 *n*-octanal:4.2 *n*-hexanal:0.6 *n*-decanal:1.6 (*Z*)-4-decenal:1.5 hexanoic acid:0.7 octanoic acid. This ratio was based upon previous published chemical analyses (Douglas et al., 2001, 2004), and it represents an average ratio of aldehydes in the crested auklet odourant. Each aldehyde volatilizes at a different rate and the relative amounts are variable. However, all of these compounds are consistently present in the crested auklet's odourant together with (*Z*)-4-dodecenal and (*Z*)-6-dodecenal (Douglas et al., 2001, 2004; Douglas, PhD dissertation (cited earlier), 2008a; unpublished data). Studies that failed to find these compounds may have had problems with sampling methods, sample preservation, or identification of these compounds (Douglas, 2008b).

2.2. Chemical analyses

A sample of the integument used in experiment 1 was dissected under a microscope using a clean scalpel after plucking all contour and downy feathers with clean forceps. Tissue samples were placed in a borosilicate glass vial with a Teflon-lined cap and extracted in methanol with 3% unsymmetrical dimethylhydrazine. This treatment accomplishes rapid derivatization of aldehydes to dimethylhydrazones. The vial was sealed with a vapour seal and frozen until chemical analyses. This method is useful for achieving chromatographic separation and mass spectral characterization of aliphatic aldehydes (McDaniel and Howard, 1985). The *N*,*N*-dimethylhydrazones of the aldehydes are more stable than the aldehydes. This derivatization is necessary to separate chromatographic peaks for isomers such as the dodecenals that occur in the crested auklet's odourant (Douglas et al., 2004). Methods for chemical analysis were very similar to those reported in Douglas (2008a). Briefly, gas chromatography–mass spectrometry was carried out in the selective ion monitoring (SIM) mode using a HP5890 Series II Gas Chromatograph equipped with a 20 m × 0.25 mm, 5% phenyl siloxane column (Alltech, USA), and a HP5972 Series Mass Selective Detector. Retention time and ion abundances were obtained in EI mode from replicate standards (listed below) at different concentrations. Two analytes, (*Z*)-4-dodecenal and (*Z*)-6-dodecenal, are not available commercially. These compounds were identified from unique mass spectra reported in Douglas et al. (2004) and from my own reference samples and data.

Standards were prepared by serial dilutions in methanol. Derivatisation of standards with unsymmetrical dimethylhydrazine at 3% concentration in methanol was accomplished at the second dilution in the series. Undecenal (97% Undecyclenic Aldehyde, ACROS Organics, C.A.S. 112-45-8) was used as the internal standard. Precise quantities of standards were measured with an Eppendorf Pipette (Model 4710), with calibration as described in Douglas (2008a). The following chemicals were used for chemical analysis and bioassays: octanal 99% (C.A.S. 124-13-0), hexanal 96% (66-25-1), decanal 95% (11231-2), heptanal 95% (111-71-7), hexanoic acid 99+% (142-62-1), octanoic acid 99% (124-07-2) all by ACROS Organics; Z-4-decenal, 95% (21662-09-9) by Lancaster Synthesis, and nonanal, 97% (124-19-6) by Alfa Aesar. Standards for possible trace compounds used in chemical analysis were 1-octanol 99% (111-87-5) by Avocado Research Chemicals Ltd., undecyclic aldehyde 97% (112-44-7) by ACROS Organics, and tridecanal 94% (10486-29-8). Methanol (67-56-1, HPLC grade, ACROS Organics) was used as the solvent for extractions and standards. The chemical agent used to derivatise aldehydes was unsymmetrical-dimethylhydrazine, 99% (57-14-7, ACROS Organics).

2.3. Social behavior

I tested the hypothesis that crested auklets and least auklets do not differ with respect to group size, interindividual spacing and rates of contact. I viewed crested auklets and least auklets from hidden vantages above steep rock talus slopes on Sivuqaq Mountain, St. Lawrence I., Alaska, where the two species nest sympatrically. Counts were made on June 25, 26, 27, 28, 30th, 2005 during morning activity periods, when crested and least auklets are active on the colony surface. I operated tally counters in each hand while I made observations with 10 × 40 binoculars. After each observation I recorded data in a waterproof field data book. I alternated counts between crested and least auklets. Interindividual spacing was quantified by randomly selecting an individual bird and estimating its nearest neighbour based upon body length. I quantified group size by sequentially selecting rocks where auklets stood or perched and then selecting the first bird that came into view on that rock to begin counts. Inclusion in a social neighbourhood required that all individuals had to be within 1.5 body lengths of another conspecific. At a spacing of 1.5 body lengths crested and least auklets are within approximately three paces of making contact with each other. There is a strong potential that they will engage in social or agonistic interactions. Contact can be precipitated when another bird arrives or alights on the same rock, causing perched birds to adjust their positions. Crested auklets that are in close proximity can also run into each other if alarmed.

Conspecific contact rates were determined by 1 min focal observations. Birds were randomly selected by directing binoculars below the landing or socializing rock and then selecting the first individual that came into view as the binoculars were raised. The number of times conspecifics made contact with a focal bird was counted for 1 min. Each instance of contact represented an opportunity for louse transmission because feathers of individual birds came in contact with each other. Results were combined across days and analysed with the independent samples *t*-test after applying Levene's test for equality of variances (IBM SPSS Statistics 19).

2.4. Ectoparasite loads

Nine crested auklet chicks were captured as they fledged at the Main Talus colony at Buldir I., on July 31, August 2 and 6, 2003. These specimens were collected and euthanized as voucher specimens for a marine ecology study. Each specimen was placed in an individual clean Ziploc bag to avoid transfer or loss of lice. Subsequently each specimen was fumigated with carbon dioxide in a white plastic jar and then ruffled over white paper to remove lice.

Specimens were returned for additional fumigation until no additional lice could be obtained. The methods that I used were similar to Visnak and Dumbacher (1999), but in this study the whole specimen was fumigated, including the head. The combination of fumigation with CO_2 and post-mortem feather ruffling is effective at removing the majority of lice (Clayton and Drown, 2001). The louse load for each fledgling was tabulated. Louse loads on fledgling crested auklets were presumed to reflect louse transmission from their own parents. Crested auklets vigorously defend their nest crevices (Fraser et al., 2002), and therefore chicks are only likely to have contact with their own parents in their nest crevice.

3. Results

3.1. Bioassays

Auklet lice exposed to volatiles from odourant secreting tissues of a male crested auklet became moribund within a few seconds. After 16 h one louse began making very slow jerky movements of appendages. A second louse was non-ambulatory with spasmodic twitching after 16 h, but it began to make very slow jerky movements of appendages after 22 h. These two lice subsequently became ambulatory and survived until 64 h elapsed time. The other five lice exposed in the same manner remained moribund for 96 h. After 96 h all moribund lice were probed with a fine camel hair brush while observed under a microscope, but no response was noted. All lice exposed in the same manner to control tissues of the same bird survived with normal and active behaviour for at least 64 h, and all except one of these control lice survived with normal activity for at least 96 h.

Auklet lice became moribund when exposed to volatiles from synthetic analogues of auklet odourants. In experiment 2 lice became moribund in 3–12 min depending upon dose (Fig. 1). Lice remained moribund and did not respond to probing with a fine camel hair brush after 18 h. All experimental lice were presumed dead. Dose-dependent responses were noted for auklet lice at very low concentrations but impairment was more gradual and less uniform. Lice transitioned through all stages from normal activity to reduced ambulation and immobilisation. Some lice fell off feathers while others became rigid, with appendages clasped tightly around feather barbs. Elapsed time until onset of impairment varied with dose and was incrementally slower at lower doses. In experiment 3 auklet lice became moribund after 14 min (1.0% treatment), non-ambulatory and immobilised after 34 min (0.5%), and non-ambulatory and immobilised after 41 min (0.1%). In 0.5% and 0.1% treatments a louse remained non-ambulatory with spasmodic twitching for 5.75 and 6.25 h, respectively. No lice recovered at any stage after exposure. Lice probed with a fine-haired camel brush after 46 h did not respond and were presumed dead.

Pigeon lice manifested dose-dependent responses to crested auklet odourant. Activity of lice in experimental treatments was reduced and impaired compared with control treatments. Pigeon lice exposed to 1.0%, 0.5%, 0.1% and 0.05% concentrations were impaired in 1, 2–6, 7.5–11 and 24 h, respectively. By contrast all lice in control treatments had normal activity at all intervals and continued so after 25.5 h. At 1.0%, pigeon lice became non-ambulatory or immobilised within 1 h, but none became moribund until 12.75 h. At the intermediate doses (0.5%, 0.1%) the effects were less. At 0.5% all pigeon lice remained immobilised or had reduced ambulation combined with spastic or jerky movements of appendages for up to 33 h, but none became permanently moribund. In the 0.1% treatment the lice were impaired with reduced ambulation combined with spastic or jerky movements of appendages. This began to wear off for some of the lice by 28 h, but some remained impaired for up to 33 h. At the lowest dose (0.05%) no lice became moribund until 31 h. Differences in body size could have contributed to variability in rates of impairment. Pigeon wing lice (*Columbicola*) are larger than pigeon body lice (*Campanulotes*), and both species were assigned to all treatments and replicates. Pigeon wing lice are also significantly larger than lice that occur on auklets (J. Malenke, personal communication).

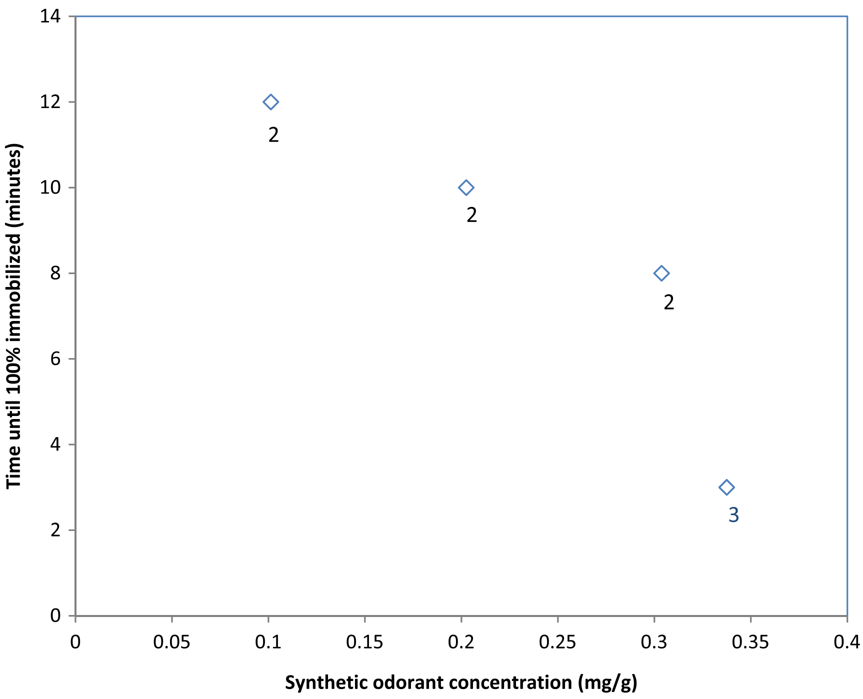

Fig. 1. The elapsed time until immobilisation of all lice in a sample is shown versus the concentration of aldehydes in a treatment. The number of replicate samples for treatments is indicated by a numeral. Lice obtained from crested auklets (genera *Austromenopon*, *Quadraceps*, *Saemundssonia*) were used in these experiments.

3.2. Chemical analyses

Chemical analyses showed that constituents of the crested auklet odourant were present in tissues where bioassays were conducted. These constituents were hexanal, octanal, decanal, (*Z*)-4-decenal, (*Z*)-4-dodecenal, and (*Z*)-6-dodecenal. I identified these compounds from retention time of chromatographic peaks and mass spectra of their *N*,*N*-dimethylhydrazones. Possible trace constituents reported in Hagelin et al. (2003) were not found.

3.3. Social behaviour

Crested auklets had larger group sizes than least auklets, $t_{(172)} = 6.078$, $P < 0.001$) and closer interindividual spacing $t_{(31.7)} = -3.88$, $P < 0.001$. Crested auklets also had more frequent contact rates with conspecifics than did least auklets $t_{(52)} = 3.837$, $P < 0.001$. Group sizes among crested auklets (mean = 2.79, S.D. = 2.44, $n = 140$) were relatively larger than least auklets (mean = 1.46, S.D. = 0.991, $n = 195$) by a factor of 1.9. Least auklets were more dispersed on the colony surface compared with crested auklets; crested auklets had relatively closer interindividual spacing based on conspecific body lengths (mean = 0.81, S.D. = 1.44, $n = 22$ versus mean = 3.54, S.D. = 3.04, $n = 23$). Direct contact between crested auklets (mean = 3.95, S.D. = 5.44, $n = 41$) occurred more frequently than among least auklets (mean = 0.44, S.D. = 2.19, $n = 41$).

3.4. Ectoparasite loads

Lice were normally distributed on fledglings (Shapiro–Wilk = 0.92, $P = 0.4$, df = 9), but louse loads were highly variable, ranging from as few as three to as many as 195 (mean = 90, S.D. = 70, $n = 9$). The median value was 66. Many lice occurred on the heads of some chicks. The chick with the second highest louse abundance had 180 lice, and 40 were obtained from the head, face and under the chin.

4. Discussion

The crested auklet's aldehyde odourant impaired lice at concentrations similar to those that occur in free-living crested auklets. Lice exposed to volatiles from nicks in the integument were killed or paralysed after brief exposure. By contrast, lice exposed to nicks in the flank of the same specimen were not affected. Samples of the integument used for the experimental treatment in this bioassay contained hexanal, octanal, decanal, (*Z*)-4-decenal, (*Z*)-4-dodecenal and (*Z*)-6-dodecenal. All of these compounds are volatile and they are the only substances that were present in the integument that could have caused mortality and paralysis in the lice. Five lice exposed to these volatiles did not recover and were presumed dead. Two lice gradually began to regain mobility after 16 and 22 h, respectively. This shows that the crested auklet odourant can paralyse lice. However, it does not demonstrate the magnitude of effects, because the rate of aldehyde flux from integument to plumage is not known. Interestingly, the crested auklet specimen used in this experiment was obtained during chick rearing, and in a previous study the crested auklet with the highest emissions of octanal was measured during chick rearing (Douglas, 2006). Some authors have suggested that the crested auklet's citrus-like odourant senesces or diminishes significantly earlier in the breeding season (Hagelin et al., 2003; Hagelin and Jones, 2007; Hagelin, 2007; Sealy, 2006). These authors based their assumptions in part on their own sensory perceptions of how abundant the citrus-like odour seemed to be in the environment. However, the citrus-like scent of crested auklets can sometimes be detected during early chick rearing at distances up to 1.2 km from crested auklet colonies.

Lice exposed to volatiles from blends of synthetic aldehydes manifested a sequence of impaired behaviours similar to neuroactive insecticides (e.g., pyrethroids and nicotine, Corbett et al., 1984). Dose-dependent effects were noted for a range of concentrations similar to those that can occur in the plumage of free-living crested auklets (Fig. 1). Effects were noted even at very low concentrations similar to those that can occur in the plumage of free-living crested auklets during winter. Effects at lower doses took longer to manifest and the time dependence of dose response noted in this study suggests that impairment could be related to the rate at which aldehydes diffuse into louse spiracles. Lice exposed to the control treatment were unaffected; the same quantities of control substance were present in experimental and control treatments. These assays did not exactly replicate conditions in nature. Exposures were limited to a volatile fraction, but lice on crested auklets could come into contact with aldehyde secretions and other compounds that could have synergistic effects, e.g., hexanoic acid and octanoic acid. Furthermore, while the treatments in these experiments were single exposures, lice could encounter multiple exposures from the repeated anointing behaviours of crested auklets. In these experiments lice were not able to escape volatiles but in nature lice may be able to move away from volatiles. Some species of lice aggregate on the head, ostensibly because birds cannot self-preen in these areas (Clayton and Drown, 2001; Johnson et al., 2005). High concentrations of aldehydes occur on the back of the head and neck coincident with alloanointing behaviour (Douglas, 2008a); this could repel lice, protecting these vulnerable areas from feather damage and the increased thermal conductance that could result. As Weldon (2010) argued, vertebrate chemical defenses could include aposematic semiochemicals that cause ectoparasites to avoid certain body regions.

To my knowledge this is the first study to clearly document that the crested auklet's odourant has dose-dependent effects against avian lice, similar to neuroactive insecticides. In the context of host–parasite interactions the exposure may stun or debilitate the parasite. When aldehydes are perfused into crested auklet plumage during alloanointing the resulting concentrations (1.29 mg/g total aldehydes on head, 280 μg/g total aldehydes in mantle plumage, Douglas, PhD dissertation (cited earlier), 2008a) can be sufficient to repel, impair, stun or kill lice. Previous bioassays have suggested that the crested auklet odourant can repel, impair and kill ectoparasites, depending upon dose (Douglas et al., 2004, 2005b; Douglas, 2008a). Blum (1981) hypothesised that aldehydes in insect chemical defenses can overwhelm chemosensory receptors. Eliyahu et al. (2012) showed that aldehydes in heteropteran chemical defenses can have paralytic effects on southern fire ant (*Solenopsis xyloni*) workers. Volatile aldehydes could impair lice by diffusing into spiracles and reacting with respiratory membranes, nerve cells and muscle cells. These effects are consistent with toxicity information described in manufacturer's material safety data sheets. All aldehydes are strong irritants and they affect mucous membranes (Schauenstein et al., 1977). Aldehydes can react with organic molecules and interfere with cell signaling and cell functions (Schauenstein et al., 1977; Esterbauer et al., 1991; O'Brien et al., 2005; Hill et al., 2008). Cytoxicity of aldehydes depends upon their electrophilicity (Niknahad et al., 2003; Hill et al., 2008), specific chemical properties (Schauenstein et al., 1977), and the metabolism of the affected cell (Chan et al., 2008). Aldehydes in the crested auklet odourant are aliphatic and aliphatic aldehydes are electrophilic (Appel and Mayr, 2011). Conjugated aldehydes in the crested auklet odourant are likely more electrophilic than saturated aldehydes, although probably not as cytotoxic as α,β-unsaturated aldehydes.

The crested auklet odourant is a chemical defense that could complement mechanical defenses. Preening is a primary defense that birds utilise against lice (Cotgreave and Clayton, 1994; Clayton et al., 2005, 2010). During preening, birds move their beaks very rapidly and this causes decapitation, loss of appendages and laceration of the louse exoskeleton (Clayton et al., 2005). Lice attempt to escape preening by rapid locomotion through plumage but lice impaired by fumigation would be slower to escape. Allopreening may accompany alloanointing in crested auklet social behaviour (Douglas, 2008a). During alloanointing crested auklets rub their accessory bill plates over their partner's interscapular area (Douglas, 2008a). Relatively high concentrations of aldehydes are detectable on an accessory plate of the lower mandible when crested auklets are actively alloanointing (3.8–29.5 $\mu g/cm^2$; Douglas, PhD dissertation (cited earlier), 2008a). Crested auklets rub their bills through their partner's plumage. This behaviour is similar to avian anointing behaviours in which a concentrated chemical repellent is rubbed over plumage (Ehrlich et al., 1986; Clayton and Vernon, 1993; Weldon and Carroll, 2006; Clayton et al., 2010). Even if lice are only slowed temporarily it could make them more vulnerable to mechanical defenses. I have inspected the head and neck plumage of crested auklets and found lice that have been decapitated and otherwise fragmented. This could have resulted from allogrooming, which is directed around the face and corners of the mouth in crested auklets. These may be focal sites for ectoparasite infections, because crested auklets often scratch these areas with their claws. Interestingly, in a previous study the crested auklet with the lowest octanal emissions had 14 *Ixodes uriae* ticks attached at the corner of the mouth while the remainder of the sample had few or none (Douglas, 2006).

Characteristics of social networks have been used to estimate transmission of infectious disease (McCallum et al., 2001; Mossong et al., 2008; Ferrari et al., 2011). In this study contact rates and the apparent density of the social neighbourhood were used to compare the relative risk of louse transmission among sympatric congeners – crested auklets versus least auklets. Contact rates were higher in crested auklets than least auklets. Social group size and interindividual spacing were also greater for crested auklets. Crested auklets may have more frequent contact with more social contacts and this could result in higher rates of louse transmission. This may help to explain why crested auklets can have higher abundances of lice than least auklets (Douglas et al., 2005a). Sociality could increase susceptibility to louse infections, consistent with the principle that higher rates of parasitism are an expected cost of sociality (Duffy, 1991; Loye and Carroll, 1995). In this study louse loads on crested auklet fledglings conformed to a normal distribution, rather than the negative binomial distribution often characteristic of parasites (Price, 1980). There was high variance in louse loads, suggesting that there were differences in the rates of louse transmission between adults and chicks.

Prevalence and intensity of louse infections apparently vary among and within crested auklet populations. Some studies have found a very low prevalence and intensity of louse infestations (Douglas et al., 2004; Douglas, 2006, 2008a), while other studies have documented relatively high prevalence and incidence of louse infestations in crested auklets (Douglas et al., 2005a). Clayton et al. (1992) argued that parasite-mediated selection acts upon variance in the relative fitness of hosts. Crested auklets may differ in the amount of aldehydes that they emit (Douglas, 2006). Steroid hormones could regulate the productivity of secretory tissues, since octanal emissions covary with circulating concentrations of progesterone (Douglas et al., 2008). The total amount of aldehydes that are emitted could also be related to the number and size of secretory wick feathers (Douglas, 2008a). Variability in these novel anatomical features could be genetically determined, and hence variability in aldehyde emissions could be linked to variance in relative fitness. Similar to other chemical repellents, the crested auklet's aldehyde odourant has a limited efficacy in time and space. Many volatile repellents wane in effectiveness after 1 h (Dautel et al., 1999), and their radius or range of efficacy is limited to approximately 4 cm from the application site (Mailbach et al., 1974). Lice have a generation time of less than 1 month (Marshall, 1981). If lice are able to persist in even small reservoirs within the host population they can become reestablished utilising the social network of their hosts when conditions are favourable. This may explain how crested auklet chicks can have high abundances of lice even at colonies where the incidence of lice is very low among adults (Douglas et al., 2004).

If crested auklets select mates which emit more aldehydes they may obtain greater protection from ectoparasites for themselves and their offspring. Crested auklets that secrete more aldehydes in plumage may have greater resistance against ectoparasites and as a result may transmit fewer lice. This is relevant to models of parasite-mediated selection. According to the model proposed by Clayton et al. (1992) the distribution of parasites among hosts and the average effects of individual parasites on hosts governs parasite-mediated selection. Average effects of socially transmitted parasites could be particularly significant for louse transfers from parent to offspring. When crested auklets transmit a louse to offspring there is a strong possibility that it is a female *Quadraceps aethereus*, because these tend to be the most abundant lice on adult crested auklets during chick rearing (Douglas et al., 2005a; Douglas, PhD dissertation (cited earlier), p. 208). Transfer of a gravid female louse from a parent to offspring could represent a large effect because it represents infection of a new host that is multiplied when the female lays eggs. Birds with higher louse abundances spend more time on self-maintenance (Cotgreave and Clayton, 1994). High louse abundances can reduce feather mass to such an extent that thermal conductance increases (Booth et al., 1993). Marine diving birds cope with a higher coefficient of thermal conductivity than has been reported for free-ranging pigeons (Booth et al., 1993). This is because the thermal conductivity of seawater is greater than air (Stahel and Nicol, 1982; Ellis and Gabrielsen, 2002). For example, the thermal conductivity of water is 0.587 W/m K for 30‰ salinity at 10 °C (Sharqawy et al., 2010), compared with 0.0247 W/m K for air at 6.85 °C (280 K, 1.00 bar; Stephan and Laesecke, 1985). Rates of convective heat transfer in water can be orders of magnitude greater than air because the thermal boundary layer in water is less than in air and only a small amount of movement is necessary to disturb it (Stahel and Nicol, 1982). Louse infestations that result in loss of feather insulation and increased thermal conductance could compromise physiological condition and reduce viability, particularly for juvenile crested auklets that may be less efficient at foraging and self-maintenance.

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