

An Extraordinary Host-Specific Sex Ratio in an Avian Louse (Phthiraptera: Insecta)—Chemical Distortion?

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Environ. Entomol. 1–6 (2015); DOI: 10.1093/ee/nvv073

ABSTRACT Distortions of sex ratios and sexual traits from synthetic chemicals have been well documented; however, there is little evidence for such phenomena associated with naturally occurring chemical exposures. We reasoned that chemical secretions of vertebrates could contribute to skewed sex ratios in ectoparasitic insects due to differences in susceptibility among the sexes. For example, among ectoparasitic lice the female is generally the larger sex. Smaller males may be more susceptible to chemical effects. We studied sex ratios of lice on two sympatric species of colonial seabirds. Crested auklets (*Aethia cristatella*) secrete a strong smelling citrus-like odorant composed of aldehydes while a closely related congener the least auklet (*Aethia pusilla*) lacks these compounds. Each auklet hosts three species of lice, two of which are shared in common. We found that the sex ratio of one louse species, *Quadraceps aethereus* (Giebel), was highly skewed on crested auklets 1:69 (males: females), yet close to unity on least auklets (1:0.97). We suggest that a host-specific effect contributes to this difference, such as the crested auklet's chemical odorant.

KEY WORDS sex ratio distortion, lice, seabird, aldehyde

Theories of sex ratios and allocation have been successful in predicting and explaining causes of variation, but broad taxonomic variation in sex ratios remains an intriguing problem (West et al. 2002). Distortion of the 1:1 sex ratio has been documented in animals across the taxonomic spectrum (e.g., ectoparasitic insects, Marshall 1981, Wheeler and Threlfall 1986, Clayton et al. 1992, Shutler et al. 2003, Pap et al. 2013; reptiles, Freedberg and Wade 2001; birds, Clutton-Brock 1986, Donald 2007, Rutkowska and Badyaev 2008, Hill et al. 2013; mammals, Clutton-Brock and Jason 1986, Smith et al. 1996, Fisher 1999, Cameron 2004; fish, Morgan and Trippel 1996, Lange et al. 2011). Skewed sex ratios have been theorized to confer an evolutionary benefit, such as when ecological conditions result in local mate competition (Hamilton 1967, Read et al. 1995, Pap et al. 2013) or by selecting for parental control of sex ratio in accordance with parental investment (Trivers and Willard 1973) and in response to environmental factors (Craig et al. 1992). However, in many cases, a skewed sex ratio is not an adaptive response. Postzygotic female-biased sex ratios occur in natural populations of ectoparasitic insects because the males are generally smaller, more active, and are often exposed to greater risk of mortality (Marshall 1981, Wheeler and

Threlfall 1986, Brooke and Nakamura 1988, Clayton et al. 1992).

Skewed sex ratios among many invertebrates result from the presence of sex ratio distorters (SRD), defined by Stouthamer et al. (2002) as heritable microbial or genetical elements that promote their own transmission through distortion of the host's sex ratio (Stouthamer et al. 1990; Werren 1991, 1997; Werren et al. 1995; Hurst et al. 1997b; Stouthamer et al. 2002; Kyei-Poku et al. 2005; Covacin and Barker 2007; Weinert et al. 2007). This may manifest as male-biased mortality, feminization of males, inducement of parthenogenesis, or a cytoplasmic incompatibility which happens to discriminate against progeny of one sex (Hurst, 1991; Hurst et al. 1997a,b; Jiggins et al. 1998, 2000; Stouthamer et al. 2002). Some SRD are ubiquitous among arthropods. *Wolbachia* infections are widespread among insects (17–76% of insect species, Werren et al. 1995, Jeyaprakash and Hoy 2000) including chewing lice (Kyei-Poku et al. 2005, Covacin and Barker 2007). *Wolbachia* have been detected in multiple genera of Mallophaga (chewing lice) including *Quadraceps* spp. and *Saemundssonia lari* collected from herring gulls (Kyei-Poku et al. 2005, Covacin and Barker 2007).

Skewed sex ratios have also been associated with chemical exposures (Dodson et al. 1999, Peterson et al. 2001, Shutler et al. 2003, Lange et al. 2011). Chemical exposures can disrupt animal endocrine systems (Sattin et al. 1984, Eli and Nisula 1990, Garey and Wolff 1998, Go et al. 1999, Kim et al. 2004, Hayes et al. 2011), altering sexual traits (Reeder et al. 1998), and causing skewed sex ratios (Dodson et al. 1999, Peterson et al. 2001, Lange et al. 2011). Shutler et al. (2003) found evidence for male-biased mortality of avian fleas in tree

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swallow nests that had been treated with insecticide and diatomaceous earth. Skewed sex ratios induced by chemicals from anthropogenic sources have been described, but there seem to be few examples of skewed sex ratios from naturally occurring chemical exposures.

In this report, we are especially interested in the potential for multiple factors to work together to alter the sex ratio in one population of parasite, but not in conspecifics, infesting sympatric hosts. We reasoned that chemical secretions of vertebrates could have different effects within populations of ectoparasitic insects if there are differences in exposures or differences in susceptibility. For example, among chewing lice, males are usually smaller than females. Differences in body size or segregation on the host's body could result in differences in exposures and rates of mortality. We compared the sex ratios of lice removed from crested auklets (*Aethia cristatella*) and least auklets (*Aethia pusilla*) captured in the same mist net set, in the same colony on the same dates. Some of the same species of lice occur on both species of auklets (Douglas 2005a). Direct feather to feather contact permits transmission of lice between birds (Hillgarth 1996, Brooke and Nakamura 1998, Lindholm et al. 1998, Daralova et al. 2001), and such incidental contact can occur on the crowded colonies. Crested auklets emit saturated and monounsaturated aldehydes (Douglas et al. 2001, 2004; Hagelin et al. 2003; Douglas 2006, 2008), and these aldehydes are secreted from specialized gland-like wick feathers in the integument (Douglas et al. 2001; Douglas 2006, 2008). Douglas et al. (2001) proposed that the crested auklet's odorant functions as a signal of mate quality and repels ectoparasites, linking mate quality, vigor, and resistance to parasites. The aldehydes that comprise the crested auklet's odorant are repellent to ectoparasites (Douglas et al. 2004, 2005b) and impair lice and ticks in a dose-dependent manner (Douglas et al. 2004, Douglas 2008) similar to neuroactive insecticides (Douglas 2013). These compounds have not been found in least auklet's plumage (H. D. D., unpublished data). We tested the hypothesis that population characteristics of lice on crested auklets and least auklets would differ.

Materials and Methods

We captured breeding adult crested and least auklets (crested auklet, $n = 21$; least auklet, $n = 25$) in a single mist net set at the western end of an auklet colony near Cape Myaaghee, St. Lawrence Island, AK, August 4–5, 2001. One of us, H. Douglas, delineated this subcolony on July 15, 2009 with a GPS unit and estimated its areal extent at $5.9286 \times 10^5 \text{ m}^2$; it is part of a larger colony that extends along cliffs from 10 to 16 miles east of the village of Savoonga (minimum population 1 to 3×10^6 crested auklets, H. Douglas, unpublished report). The median egg hatching date for crested auklets ($n = 44$) and least auklets ($n = 37$) at this colony was August 2 in 2001 (Gall 2004). Auklets were immediately removed from the mist net and placed inside individual paper bags to avoid loss of lice or transfer of lice between individuals. Subsequently the birds were

sacrificed for this study and other studies by cardiac compression. Each specimen was sealed inside the paper bag and placed inside an individual ziploc plastic bag. The specimens were shipped frozen to the Clayton laboratory at the University of Utah. Louse loads were quantified using the body washing method of Clayton and Drown (2001).

We characterized louse sex ratios, distributions, abundances of adults and nymphal stages, and abundances of putative generalist and specialist lice species. We expected that louse distributions could depart from normality based on assumptions in the literature (Rékási et al. 1997, Rózsa et al. 2000), although differences in host sociality could affect louse distributions (Douglas 2013). Lice were tabulated for each specimen and identified to species. We tested for normality of louse distributions on hosts with the Shapiro–Wilk (S-W) and the Kolmogorov–Smirnov (K-S) tests (with Lilliefors Significance Correction). The former test is assumed to be more powerful, but it is less reliable when there are tied data in the distribution (Zar 1999), which was true of our data. Mean abundances and standard deviations were characterized for each louse species, and we tested for differences in mean abundances of louse species (*Quadraceps* vs. *Saemundssonina*) with a Wilcoxon matched-pair signed-rank test.

Louse populations from each host species were then characterized as adults versus nymphs, and the adult lice were further classified by sex (nymphal lice cannot be sexed). Damaged lice were excluded from sexing. R.D. Price reviewed our developmental and sex classifications, and we followed his recommendations where discrepancies occurred. Sex ratios of adults were determined by pooling lice across individual crested auklets and least auklets. We used the Pearson's correlation to test for relationships between the numbers of adults and nymphs on hosts. Statistical analyses were performed with IBM SPSS Statistics version 22.

Results

The putative specialist lice, *Saemundssonina* spp. had similar male-biased sex ratios on crested and least auklets, while sex ratios of the putative generalist, *Quadraceps aethereus*, diverged considerably between hosts. On crested auklets, for *Q. aethereus*, the sex distribution was 3 males: 207 females (ratio of 1: 69); for *Saemundssonina wumisuzume* (Uchida), the distribution was 63 males: 38 females (ratio of 1:0.60), and for *Austromenopon nigropleurum* (Denny), the distribution was 6 males: 6 females (ratio of 1: 1). On least auklets, for *Q. aethereus* the sex distribution was 31 males: 30 females (ratio of 1: 0.97); for *S. boschi* (Price, Palma, Clayton), the distribution was 23 males: 17 females (ratio of 1: 0.74). No *A. nigropleurum* adults were found on least auklets, and so no comparison of sex ratios was possible for this louse species.

Lice did not have normal distributions on hosts, and abundances of adults and nymphs were correlated on hosts. None of the louse species were normally distributed on least auklets ($P < 0.001$ S-W, $P < 0.05$ K-S) or

crested auklets ($P < 0.01$ S-W, $P < 0.05$ K-S). Abundances of adult and nymphal lice were correlated for all species, with the lowest correlation being *Q. aethereus* on crested auklets ($r = 0.6$, $P < 0.05$). Correlations were the same for *S. wumisuzume* and *A. nigropleurum* on crested auklets ($r = 0.7$, $P < 0.001$, $n = 21$) and the same for *Q. aethereus* and *S. wumisuzume* on least auklets ($r = 0.8$, $P < 0.001$, $n = 25$). The only significant correlation among louse species was for nymphs of *Q. aethereus* and *A. nigropleurum* on crested auklets ($r = 0.7$, $P < 0.001$, $n = 21$). This general pattern was evident in the raw data. Crested auklets with few or no *A. nigropleurum* also tended to have fewer *Q. aethereus*.

Abundances of putative generalists and specialists differed. *Q. aethereus* were the most numerically abundant lice on crested auklets, totaling 376 compared to 217 *S. wumisuzume*, but this difference was not significant ($T_{0.05(2),21} = -1.812$, $P = 0.070$). The abundances of *Q. aethereus* and *S. boschi* were very similar on least auklets (101 vs. 91, $T_{0.05(2),25} = -0.328$, $P = 0.743$). On crested auklets, the mean abundances of lice (adults + nymphs) were—*Quadraceps*, mean = 17.9, SD = 17.3; *Saemundssonina*, mean = 10.3, SD = 8.1; *Austromenopon* mean = 2.8, SD = 4.2. On least auklets, the mean abundances of lice were—*Quadraceps*, mean = 4.0, SD = 6.2; *Saemundssonina*, mean = 3.6, SD = 4.1; *Austromenopon* mean = 0.5, SD = 0.6.

Discussion

We compared some population characteristics of lice on two closely related species of seabirds that nest sympatrically in a large colony (10^6 individuals) on St. Lawrence I., AK. One species, the crested auklet, is known for its strong plumage odorant (Douglas et al. 2001, 2004; Hagelin et al. 2003; Douglas 2006, 2008, 2013) while the least auklet lacks this odorant (H. D. D., unpublished data). Among the louse species present on the two auklet species, *Q. aethereus* had highly skewed female-biased sex ratios on crested auklets, but it had a nearly equal sex ratio on least auklets. *Saemundssonina* spp. had male-biased sex ratios on both crested and least auklets. *A. nigropleurum* had an equal sex ratio on crested auklets, but was only found in the nymphal stage on least auklets. No louse species had a normal distribution. Abundances of adult and nymphal lice were significantly correlated on hosts. *Q. aethereus* on crested auklets were the most numerically abundant lice, but they had the lowest correlation between adults and nymphal stages. This may reflect greater dispersal among hosts. The median hatch dates for the auklet colonies was ~2d prior to our sampling, and so there could have been some vertical transmission of lice from adults to offspring. Comparison of putative generalists and specialists showed *Q. aethereus* was 1.7 times greater than *S. wumisuzume* on crested auklets, and 1.1 times greater than *S. boschi* on least auklets; these differences were not significant.

Many arthropod species have SRD or host relationships with microorganisms that have the potential to

distort sex ratios (Stouthamer et al. 1990, Werren 1991, Werren et al. 1995, Hurst et al. 1997b, Werren and O'Neill 1997, Stouthamer et al. 2002, Kyei-Poku et al. 2005, Covacin and Barker 2007, Weinert et al. 2007). There has been no documented occurrence of *Wolbachia* in *Q. aethereus*, but the prevalence of endoparasites and symbionts may be underestimated among insects (Weinert et al. 2007); novel microbial SRD–host relationships may await discovery. All else being equal, a *Wolbachia* strain(s) in *Q. aethereus* would likely infect populations on both crested and least auklets, because they occupy geographically and temporally overlapping breeding colonies and occasionally come into direct contact with each other. However, highly skewed sex distortion of this louse was only evident on crested auklets. Indeed, this discrepancy across *Q. aethereus* populations makes it difficult to find a single explanation for the sex ratio patterns of these auklet lice.

It is possible that there is an interaction between potential sex ratio biasing factors affecting *Q. aethereus* and the different characteristics of its avian host species. Chemical exposures can affect the microbiomes of insects (e.g., Pettis et al. 2012), and this has not yet been explored for *Q. aethereus*. The unique chemical environment of the crested auklet's plumage could have selected for a specialized host symbiont relationship, and that could result in a separate deme of *Q. aethereus* on crested auklets. Hom and Murray (2014) have shown mutualistic relationships can arise spontaneously when favored by environmental chemistry (fungal and algal cells). Some endosymbionts can enhance survival of their insect hosts (Oliver et al. 2003, Moran et al. 2008, Oliver and Moran 2009, Xie et al. 2010). Symbionts can also promote reproductive and ecological isolation of the insect host population (Moran et al. 2008) and might have divergent effects within insect populations if the infection is more benign in one sex.

Chemical exposure may have contributed to skewed sex ratios through male-biased mortality and delayed development. The males of many ectoparasitic insects are smaller, more active and often exposed to greater risk of mortality (Marshall 1981, Clayton et al. 1992, Shutler et al. 2003), resulting in female-bias. Pesticide exposure has been shown to cause delayed development in insects with differential effects for males and females (Bossard et al. 2000, Shutler et al. 2003). Naturally occurring volatile chemicals emitted from biologically active plants in birds' nests have been linked to reduced emergence of feeding instars of mites, which could be the result of delayed development (Clark and Mason 1988).

There could be interactions between chemical exposures and other factors such as host body size and distribution on the host. The crested auklet is three times larger than the least auklet; feathers scale with host body size and associated structural characteristics tend to influence host-specificity in chewing lice (Reed et al. 2000, Clayton et al. 2003, Johnson et al. 2005, Bush and Clayton 2006). If the smaller male *Q. aethereus* is less well adapted to the larger host and further

impaired by aldehydes in crested auklet plumage they could be more susceptible to preening and separation from the host. *Quadriceps* spp. are hypothesized to be generalists that can range over the whole body of the host, while *Saemundsson* spp. are hypothesized to specialize as head lice (based on studies of other avian hosts, Johnson et al. 2012). Different species of *Saemundsson* occur on crested and least auklets, consistent with ecological specialization. Specialization on the head can reduce the defense pressure from avian hosts. Birds are not able to preen lice from their own heads, and preening is the chief mechanical defense that birds have against lice (Clayton et al. 2003, Bush and Clayton 2006). Interestingly, while female-biased sex ratios are more common among ectoparasitic lice (Marshall 1981, Wheeler and Threlfall 1986, Clayton et al. 1992), *Saemundsson* spp. exhibited male-biased sex ratios on crested auklets (1:0.61) and least auklets (1:0.71). This suggests that *Saemundsson* spp. may be less vulnerable to preening because of their distributions on their hosts.

Theories of sex ratios and sex allocation attempt to explain why particular proportions of offspring are male or female, yet the explanation of broad taxonomic patterns persists as a major research problem (West et al. 2002). The occurrence of an extraordinary host-specific sex ratio in *Q. aethereus* presents an intriguing case. The predominant theories for sex ratio skew do not offer a compelling explanation for this unusual case. We doubt that local mate competition is a tenable explanation, as this usually arises with small isolated populations where the probability of inbreeding among individuals is high (Hamilton 1967, Clayton et al. 1992). We suspect that *Q. aethereus* on crested auklets are less likely to be isolated due to the crowded social neighborhoods in a very large colony and the frequent contact between hosts (Douglas 2013). Wolbachia-induced parthenogenesis is known for Hymenoptera but not well established beyond that group and not known for insect hosts with diploid sexual reproduction, such as lice (Huigens and Stouthamer 2003, Schilthuizen and Stouthamer 1997, Stouthamer et al. 1999). Furthermore, the parthenogenetic offspring may have reduced survival and reduced competitive ability (Huigens et al. 2004). Similarly if male-killing Wolbachia eliminated most of the males in the population prior to attaining the nymphal stage it would result in a high percentage of unmated females as has been noted in other diploid insects (Jiggins et al. 2000). Population size would be reduced but instead *Q. aethereus* appear to be numerous and prevalent on their crested auklet hosts, indeed more numerous than on least auklet hosts (Douglas et al. 2005a). There may be a host-specific effect at work that reduces the lifespan of male *Q. aethereus* relative to females, and there may be a symbiont that manipulates reproduction of *Q. aethereus* on the crested auklet host and enhances survival of female lice. These lice and their auklet hosts present a naturally complex system for future exploration of the causes and consequences of sex ratio bias.

Acknowledgments

This work was conducted in compliance with approved institutional animal care and use protocols and federal and state permits, held by H.D.D. Savoonga Native Corporation permitted land access to conduct the research, and W. Wongtittin served as our guide. D.H. Clayton helped collect and transport specimens and lice, and his lab helped to support this work. C.W. Harbison and B. Moyer provided technical assistance. R. Price identified lice and reviewed J.R.M.'s classification of lice with respect to developmental stage and sex. The Savoonga School and B. Billings helped support fieldwork. A grant from the Angus Gavin Memorial Bird Research Fund, University of Alaska Foundation, supported H.D.D. A.M. Springer helped support this work.

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Received 9 November 2014; accepted 20 April 2015.