Song Analysis Reveals a Permanent Population of the Mediterranean Lacewing *Chrysoperla agilis* (Neuroptera: Chrysopidae) Living in Central Alaska

CHARLES S. HENRY, STEPHEN J. BROOKS, JAMES B. JOHNSON, MARTA M. WELLS, AND PETER DUELLI

ABSTRACT  *Chrysoperla agilis* Henry et al. (Neuroptera: Chrysopidae) is a widespread, nomadic lacewing in the *carnea* group of cryptic species. *C. agilis* has previously been found only in the warm parts of Europe, western Asia, and a few oceanic islands. Like others of the *carnea* group, *C. agilis* is identifiable only by its unique courtship song. Recently, a population with by the *C. agilis* song was discovered in central Alaska; based on its persistence over several years and its distribution over a wide area near Fairbanks, it seems to be permanent rather than transitory. To assess the relationship of this Western Hemisphere population to *C. agilis* in the Eastern Hemisphere, we 1) analyzed its courtship song, comparing it to the Eurasian song; 2) compared larval and adult morphology of Alaskan and Eurasian specimens; 3) inferred phylogenetic relationships of Alaskan and Eurasian specimens, by using sequences from the cox2 gene; and 4) crossed Alaskan with European individuals, raising their hybrid progeny and analyzing their “hybrid” songs. Alaskan *C. agilis* generally fell within the range of variation of Eurasian individuals for all acoustic and morphological traits, and their hybrid progeny were also acoustically indistinguishable. Phylogenetically, and despite current geographical isolation, Alaskan individuals clustered with Eurasian *C. agilis* rather than with Western Hemisphere taxa of the *carnea* group. We conclude that the Alaskan population is a *bona fide* member of *C. agilis*. Examination of the geographical pattern of song variation suggests that dispersal to Alaska took place quite recently in a west to east direction, via eastern Asia and the Bering Strait.

KEY WORDS  systematics, mating signal, dispersal, biogeography

Substrate-borne courtship songs in green lacewings were first discovered over 30 yr ago, in the globally distributed species *Chrysoperla carnea* (Stephens) (Henry 1979). Since then, *Chrysoperla carnea* s.l. has been separated into >16 distinct species, each characterized by its own unique vibrational song (Henry 2006). Most of these “song species” are nearly impossible to tell apart, either morphologically or ecologically; they are truly cryptic species, comprising what is now known as the *carnea* species-group of *Chrysoperla* (Brooks 1994). Most also show broad, extensively overlapping geographic ranges, usually measured in thousands of linear kilometers (Wells and Henry 1998). For example, *Chrysoperla plorabunda* (Fitch) is found from coast to coast in North America (Henry and Wells 1990); *Chrysoperla lucasina* (Lac-
surprise us when we discovered 10 yr ago that \textit{C. agilis} had colonized the Azores archipelago, 1,600 km from the nearest land mass (Europe). Nonetheless, we were indeed amazed in the summer of 2007 to find the very same species living in central Alaska, not only under highly atypical climatic conditions but also in a different global hemisphere.

Here, we present acoustical, morphological, and molecular evidence substantiating the occurrence of this Eurasian species on the North American continent. From separate collections in 2007 and 2010, we describe the song phenotype and physical appearance of these Alaskan residents, comparing the results to previously described populations of \textit{C. agilis} from nine other localities in the Eastern Hemisphere. In addition, molecular sequence data are used to infer the phylogenetic position of the Alaskan population within the \textit{carnea} group. We then describe crossing experiments between European and Alaskan representatives of the species, documenting the reproductive compatibility of these distant populations and quantifying the phenotypic characteristics of the first-generation hybrids.

Finally, we consider alternative hypotheses for the movement of \textit{C. agilis} to Alaska. Because Fairbanks is approximately equidistant from the western limit (Azores) and eastern limit (Iran) of the previously known range of \textit{C. agilis}, the species could just as easily have dispersed to Alaska westward across the Atlantic and northern Canada or eastward across Asia and the Bering Strait (Fig. 1). We address those hypotheses using inferences from acoustical and morphological data and from known patterns of global atmospheric circulation. In addition, we examine the possibility of human introduction of \textit{C. agilis} to Alaska, through past or ongoing agricultural release of commercially-grown lacewings of indeterminate species status obtained from Eurasian or North American insectaries (Henry and Wells 2007).

Materials and Methods

Collecting, Rearing, and Identification. Adult green lacewings assignable to the genera \textit{Chrysopa} Leach and \textit{Chrysoperla} Steinmann were obtained in two separate years near Fairbanks, AK. Thirty individuals were collected in 2007 between 18 July and 7 August, and 25 were collected on 4 August 2010. Sex ratio from the field was approximately unity. The principal collecting site in both years was a grassy field at the Calypso Farm and Ecology Center (64° 50’ 22” N, 148° 07’ 52” W) in Fairbanks, but 10 of 30 individuals in 2007 were collected at the town of Delta Junction, 131 km southeast of Fairbanks. Collectors were Drs. S. Em-
Henry et al. (1999b), except that maintenance, rearing, and analysis. The protocols are unfavorable weather conditions. All living specimens were deposited as vouchers in the collection of C. S. Henry, Storrs in 2007 and 2010, only 18 males and eight females were then compared by two-tailed t-tests for independent samples. For regional comparisons of means for each song feature, we performed an analysis of variance (ANOVA) of C. agilis song data from Alaska and nine other geographical populations in the Eastern Hemisphere (Table 1 and Henry et al. 2003). Scheffé’s post hoc contrast test (Scheffé 1953) was then applied to identify significant pairwise differences among populations. In addition, a principal components analysis (PCA) was implemented to summa-

Table 1. Mean values at 25 ± 1°C of the song features of C. agilis collected from nine geographic areas, plus those of Alaska–Europe hybrids

<table>
<thead>
<tr>
<th>Carrier frequency of volleys (Hz)</th>
<th>Time measures of volleys (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>Middle</td>
</tr>
<tr>
<td>Azores (n = 16)</td>
<td>84.35 ± 4.76</td>
</tr>
<tr>
<td>S. Spain (n = 5)</td>
<td>81.74 ± 2.53</td>
</tr>
<tr>
<td>S. France (n = 10)</td>
<td>83.96 ± 3.24</td>
</tr>
<tr>
<td>Switz. (n = 10)</td>
<td>77.73 ± 4.91</td>
</tr>
<tr>
<td>S. Italy (n = 21)</td>
<td>82.38 ± 4.20</td>
</tr>
<tr>
<td>Malta (n = 4)</td>
<td>83.04 ± 7.66</td>
</tr>
<tr>
<td>Greece (n = 10)</td>
<td>71.17 ± 3.07</td>
</tr>
<tr>
<td>Israel (n = 4)</td>
<td>68.12 ± 3.51</td>
</tr>
<tr>
<td>Iran (n = 11)</td>
<td>64.97 ± 3.79</td>
</tr>
<tr>
<td>Alaska (n = 20)</td>
<td>72.26 ± 6.53</td>
</tr>
<tr>
<td>ALK s (n = 12)</td>
<td>73.31 ± 7.35</td>
</tr>
<tr>
<td>AK s s (n = 8)</td>
<td>73.70 ± 5.18</td>
</tr>
<tr>
<td>AKsEur (n = 13)</td>
<td>73.51 ± 4.53</td>
</tr>
</tbody>
</table>

Each value is the mean of the means of n individuals in the population subsample, ±SD (1 SD). All variables showed at least some significant differences among the ten geographical populations (see text). Significant differences between the sexes within Alaska (AK) are marked by asterisks (*, P < 0.01).
rize and visualize the song differences among the major geographical populations of *C. agilis* (Fig. 3).

The PCA was calculated from six song features; the seventh feature, delta, was excluded because it is derived from two other variables. Statistica version 10 (StatSoft 2010) was used for all statistical analyses in this study.

**Adult Morphology.** Nineteen Alaskan specimens (12 males, seven females) identified acoustically as *C. agilis* were examined for the state of 19 external morphological characters that might distinguish them from previously described specimens of the species from the Eastern Hemisphere (Henry et al. 2003). These characters included ground color of body; presence, extent, and color of markings on stipes, palps, gena, clypeus, frons, and postoccipital region; relative abundance and distribution of black and blond setae on pronotum; relative size of basal dilation of tarsal claw (the “claw ratio” AB/BD, see fig. 4 in Henry et al. 2002); extent to which fore wing is rounded or tapered at apex; relative width of fore wing expressed as the ratio of length to breadth at widest point; presence or absence of black markings on wing veins; orientation of the Rs crossvein with respect to the Rs vein (perpendicular or oblique, see fig. 4A in Henry et al. 2006); length and color of costal setae; length of abdominal setae; relative abundance and distribution of black and blond setae on the three distal abdominal sternites; shape and relative proportions of male genital “lip” and “chin” at apex of sternite 8+9 (see fig. 4B in Henry et al. 2006); length and color of setae clothing male genital lip; and presence or absence of dark brown stripe on pleural membrane of second abdominal segment.

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**Fig. 2.** Oscillograph (left) and sonograph (right) of the typical vibrational song of *C. agilis* from Switzerland (A) and central Alaska (B). Each song shown here comprises one volley of abdominal vibration, which in this species is synonymous with the SRU. Pure tone duration represents the initial portion of the volley, preceding the noisy ending.

**Fig. 3.** Scatterplot of the first two principal components of a PCA of six song features of *C. agilis* (see text for details). Each data point represents a single individual coded by geographical region.
Larval Morphology. We examined nine first-instar, eight second-instar, and 11 third-instar larvae of Alaskan C. agilis, all of them offspring of four lab pairings of different field-collected adults. Larvae were boiled in distilled water with a drop of liquid detergent and transferred to 70% ethanol with 5% glycerin. Specimens were viewed at 50× magnification. Head markings of selected, representative individuals were illustrated (Fig. 4) and compared with markings in specimens from the Eastern Hemisphere (Henry et al. 2003).

Molecular Affinities. DNA was extracted and 516 bp of the mitochondrial cox2 gene were sequenced for two Alaskan specimens of C. agilis by Dr. Gisella Caccone and her associates at Yale University’s DNA Analysis Facility. These sequences, together with data from several new specimens of C. agilis and C. pallida Henry et al. from Europe, were added to sequences previously assembled for 16 species of the carnea group. Outgroup taxa for the phylogenetic analyses included Chrysoperla harrisii (Fitch), Chrysoperla rufulibrasis (Burmeister), Chrysoperla comanche (Banks), Chrysoperla congrua (Walker), and Chrysopa oculata Say. A maximum parsimony (MP) tree was generated in PAUP* 4.0 by using a branch-and-bound search that retained all equally parsimonious trees (MULPARS option). Gaps were treated as missing and all characters were uniformly weighted. A 50% majority rule bootstrap of 1,000 replicates was then performed to produce the final MP cladogram. Additional phenograms were generated using minimum evolution (ME) and maximum likelihood (ML; Fig. 5) criteria, by using a GTR+Gamma model of evolution (Frati et al. 1997) and 100 bootstrap replicates.

Hybridization Between European and Alaskan Individuals. Two crosses were set up, one cross between a Swiss female and an Alaskan male (28 September 2010) and the other cross between an Alaskan female and a French male (26 September). The Swiss specimen of C. agilis had been field-collected near Agarone in late August 2010, the French specimen at Mougins, southern France, in mid-August. From 14 adult progeny of the first cross, we recorded and analyzed the songs of five males and three females; from 17 adult progeny of the reciprocal cross, the songs of three males and two females were recorded and analyzed. Means of song features for the two reciprocal crosses were compared using two-tailed t-tests for independent samples. Combined means of these hybrid populations (Table 1, last row) were compared with the parental Alaskan and European populations by using an ANOVA and post hoc contrast tests.

Patterns of Distribution. To see whether variation in song features was correlated with geographic position, we first measured the great-circle distance between each of the adjacent sites from which we had collected C. agilis over the years, by using the web-based Infoplease Distance Calculator (Anonymous 2000–2007). Arranging these nine sites in a sequence of actual geographic distances along the x-axis, we then plotted the value ± SD of each song feature for each site (Fig. 6). Hypothesizing that C. agilis could
have colonized Alaska from either the east or the west (Fig. 1), we arranged the sites in two ways, one way assuming east-to-west dispersal from Eurasia through the Azores and northern Canada to Alaska and the other way assuming west-to-east dispersal from Eurasia and the Bering Strait to Alaska. We reasoned that the graph reflecting the correct history of colonization would show a smoother, graded series of mean trait values across the geographical range of the species. Analogous plots were created for each of the principal morphological features of larvae and adults (not shown).

*C. agilis* could also have arrived in Alaska through the release of imported lacewings purchased from commercial insectaries. With the help of Dr. Derek Sikes, requests for information about such lacewing releases were distributed on the Alaska Entomology list server in December, 2010. Replies were forwarded to one of us (Henry) for compilation. Living individuals were then purchased from any extant organization that had sold lacewings in Alaska over the past 12 yr. When necessary, larvae were reared to adulthood. Adults were identified to species using the playback methods described here.

**Results**

**Song Phenotype.** In every respect, the songs of Alaskan *C. agilis* closely resembled those of *C. agilis* from sites in the Eastern Hemisphere (Fig. 2; Table 1). However, the ANOVA found statistically significant differences among the nine major geographical populations for all seven song features (Table 1). Post hoc Scheffé’s tests revealed that the Alaskan and Iranian populations differed the most from the other populations, especially in their longer periods and larger deltas (Table 1). As in the species as a whole, sexual dimorphism in Alaskan *C. agilis* was evident but slight and was manifested largely as significantly longer volley durations in females than in males ($t_{18} = 3.02, P = 0.007$).

The plot of the first two components of the PCA performed on six song features revealed no unique clustering of Alaskan individuals compared with individuals from eight other geographic populations in the Eastern Hemisphere (Fig. 3). In this analysis, PC1 and PC2 explained 71.65% of total variance.

**Adult Morphology.** At the time of collection from the field, ground color of living specimens of Alaskan
C. agilis varied from medium green to solid reddish-brown, with the majority of individuals showing an intermediate condition. These colors were retained throughout the lifespan in the lab despite the long-day photoperiod. Most of the morphological characteristics of the Alaskan specimens conformed to those exhibited in specimens of C. agilis from the Eastern Hemisphere (Henry et al. 2003). However, where there were differences in the Alaskan specimens, they are described below.

In all but one of the 19 specimens examined the stipes was entirely brown, and in two of these specimens the brown marking was faint. In the remaining specimen the stipes was marked with an apical brown spot only. The palps were marked brown in all specimens. In all specimens the gena was dark brown, and this marking was fringed with red in eight specimens. The clypeus was marked dark brown and, in all but six specimens, this marking had a red margin. In seven specimens the frons was unmarked but was margined in red in 12 specimens. The postoccipital region and vertex were unmarked in all but four specimens, which had red markings. The proportion of black (versus blond) setae on the pronotum ranged from 20 to 100%, averaging 72%. The range of the claw ratio was 2.09–2.73, and the mean ± SD (2.36 ± 0.20) was significantly different from Eastern Hemisphere specimens (2.23 ± 0.15; \( t_{43} = -2.54, P = 0.015 \)). The proportion of black (versus blond) setae on the three apical abdominal sternites ranged from 0 to 100%, averaging 25%.

**Larval Morphology.** Most of the morphological characteristics of the Alaskan larval specimens of C. agilis conformed with those described for C. agilis from the Eastern Hemisphere (Henry et al. 2003). However, where there were differences in the Alaskan specimens, they are described below.

Larvae showed limited variation in their head markings (Fig. 4): none had frontal spots, and the dorsolateral stripes varied little in width. Third-instar larvae from Alaska lacked the angular lateral expansion of the dorsolateral stripe anterior to the basolateral expansion, and 36% of them clearly exhibited a rounded mesal expansion of this stripe (Fig. 4B). Thoracic and abdominal markings were present but not prominent.

**Phylogenetic Position.** Under MP, ME, or ML criteria, the two C. agilis specimens from Alaska emerged as most closely related to each other; together, they usually clustered with the 10 species of the Eurasian clade of the carnea group, forming a large polytomy (e.g., Fig. 5). Six North American species (not including Alaskan C. agilis) comprised the second major clade. Consequently, a hypothesis of dispersal (or introduction) of C. agilis from Eurasia to North America was supported, rather than independent (convergent) origin of an agilis-like song in an existing Alaskan population of carnea-group lacewings.

**Songs of F₁, “Hybrids.”** Progeny from the two reciprocal crosses between Alaskan and European C. agilis produced song features that were statistically indistinguishable (t-tests; not shown), so we pooled data for all hybrids (Table 1, row 13). The songs of those 13 individuals were generally very similar to those of their parents. In fact, for five of the seven measured song features, hybrids exhibited values that were intermediate between their parents (compare rows 4, 10, and 13 in Table 1). For four features, an ANOVA found statistically significant differences among hybrids, parents from Europe, and parents from Alaska (period: \( F_{2,39} = 3.01, P < 0.0001; \) mid-frequency: \( F_{2,40} = 29.71, P < 0.0001; \) end frequency: \( F_{2,40} = 6.47, P = 0.004; \) delta: \( F_{2,40} = 36.25, P < 0.0001 \)). Post hoc Scheffé’s tests revealed that the features of the hybrid songs were not consistently more different from one parent than they were from the other parent. In combination, these results clearly supported inclusion of the Alaskan population within the existing Eurasian species C. agilis.

**Colonization of Central Alaska From Eurasia.** Plots of means and variance in song features by geographic position did not unequivocally support either an eastward or a westward direction of colonization for Alaska (Fig. 6). However, largely due to greater acoustical similarity between Alaskan and Iranian populations (Table 1), transitions of song features in both the temporal and frequency domains were visually more linear when it was assumed that C. agilis dispersed to Alaska from eastern Asia rather than from western Europe via the Azores (Fig. 6B and D). Larval and adult morphology showed no apparent clinal trends across the global range of the species that might allow us to conclude where within this range the Alaskan specimens might be derived.

We learned that several commercial purchases and releases of lacewings had taken place in Alaska during the last 12 yr, but only near Anchorage, far to the south of Fairbanks. In all cases, insects had been ordered from two North American companies that were still in business, March Biological in Sherwood, OB, and Arbico Organics in Tucson, AZ. We ascertained from song analysis of purchased insectary stocks that both companies had sold us C. rufulibris.

**Discussion**

**Song Phenotype.** Although some song features of Alaskan C. agilis differ significantly from those of Eastern Hemisphere individuals (Table 1), those differences are minor, and the overall form of the song cannot be mistaken for that of any other species in the carnea group (Fig. 2). This unity of song phenotype across all geographic sites is shown best in the PCA plot, where all populations overlap extensively with one another in acoustic space (Fig. 3). The complexity of the C. agilis song argues against independent origin of the same set of song attributes in different global hemispheres, in contrast to the simple song of North American C. adamsi Henry et al. that has clearly evolved convergently in an unrelated Asian species (Henry et al. 1999a).

The generally intermediate phenotype of “hybrid” songs is an expected result of crossing two variants of a species that possess very similar but measurably different courtship signals. Data from such crosses,
including all details of the courtship and mating process between European and Alaskan individuals, are completely consistent with the parents being members of the same species.

**Morphology of Adults.** Most of the morphological characters of the Alaskan specimens examined in this study fall within the range of variation exhibited by *C. agilis* specimens from the Eastern Hemisphere (Henry et al. 2003), although the Alaskan specimens tend toward the more heavily marked end of the spectrum of variation. Compared with specimens from the Eastern Hemisphere, Alaskan individuals are more likely to have red head markings, and they exhibit higher percentages of black setae on the pronotum and apical abdominal segments. In addition, claw ratio in Alaskan specimens is toward the high end of the gradient: on average, the basal dilation is narrower. However all such differences in morphology between global hemispheres are quite small.

**Morphology of Larvae.** Larvae of *C. agilis* from Alaska fall largely within the range of variation exhibited by this species in the Eastern Hemisphere (Henry et al. 2003), although the Alaskan specimens tend toward simpler and lighter markings on the head and body. In contrast to some specimens of *C. agilis* from the Eastern Hemisphere, Alaskan larvae do not have frontal spots on their head capsules, and approximately one-third of them show a small mesal extension of the basolateral stripe that has not been found in other populations of the species (Fig. 4B). Among the populations previously described, Alaskan larvae most closely resemble specimens from Sicily (fig. 4A and B in Henry et al. 2003).

**Origin of Alaskan Population.** Adult and larval morphology are uninformative with respect to directional dispersal hypotheses, and plots of song phenotypes versus geographic position provided only modest support for west to east dispersal of *C. agilis* to central Alaska (Fig. 6). However, eastward movement is consistent with the effect of the prevailing westerly winds on many vagile organisms within those latitudes (Johnson 1969, Gressitt 1974, Buden 2010, Chapman et al. 2011). Thus, we tentatively propose that *C. agilis* arrived in Alaska by dispersing from Europe through Asia and across the Bering Strait (Fig. 1). Based on extremely short branch lengths within the *carnea* group (Fig. 5), we infer that all species are very young, which in turn suggests that colonization of Alaska by *C. agilis* has been recent—certainly in postglacial times, and perhaps <1,000 yr ago. Nonetheless, the species seems to have adapted well to central Alaska’s extreme climate, including bitter cold temperatures (to −5°F) and daylengths of from 1 to 23 h. *C. agilis* is also well established there, having been collected as the most abundant summer species of *Chrysoperla* in separate years and at sites >100 km apart.

The existence of *C. agilis* in central Alaska is probably not the result of recent agricultural introduction. Mass release of lacewings has been confined to coastal Alaska rather than the Fairbanks region, and the recent suppliers of lacewing stocks for biocontrol purposes are not currently selling *C. agilis*. In addition, the Alaskan population of *C. agilis* exhibits measurably distinct phenotypic traits, specialized ecophysiological adaptations, and apparent permanence around Fairbanks. These multiple lines of evidence suggest that *C. agilis* is a dominant, persistent component of central Alaska’s lacewing fauna, which escaped the ecological constraints of its conspecifics in the milder parts of Eurasia and moved into the Western Hemisphere. Furthermore, the existence of this stable population of *C. agilis* in Alaska predicts that the species probably occurs farther east in Asia than the current known range.

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**References Cited**


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