Laboratory Hybridization Between the Introduced and the Indigenous Green Lacewings (Neuroptera: Chrysopidae: *Chrysoperla*) in Japan

HIDESHI NAKA,[1] TAKAYUKI MITSUNAGA,[2] AND ATSUSHI MOCHIZUKI[1, 3]

ABSTRACT   We report on laboratory hybridization between the indigenous *Chrysoperla nipponensis* and its close relative, *C. carnea*, which has been imported since 2001 from Germany to Japan as a biological control agent. Interspecific hybrids were obtained, and fully viable and fertile F1 and F2 generations were produced. Crosses between *C. carnea* females and *C. nipponensis* males showed 41.3% fertility, but the reciprocal cross showed only 9.5% fertility. Despite the low fertility of interspecific crosses, most F1 hybrids were fertile and were successfully backcrossed to both parental species. However, F1 males from *C. carnea* females × *C. nipponensis* males showed low fertility (zero except for 20% in one case) when crossed with any females (F1 or backcross). In the one combination resulting in 20% fertility, an extremely long preoviposition period was observed, caused presumably by a mating delay. The absence of change in hatchability, adult emergence rate, total developmental period, or deviation from the 50:50 sex ratio from parents to the F2 generation of hybrids suggests that there is no genetic incompatibility between *C. carnea* and *C. nipponensis* resulting from hybrid breakdown. Because the courtship songs of the introduced *C. carnea* and the indigenous *C. nipponensis* are quite different, interspecific mating between the two close relatives may not occur under natural conditions. It will be necessary to monitor the establishment of the introduced *C. carnea* and its possible hybrids with the indigenous *C. nipponensis* in the wild to minimize any irreversible ecological risks, such as loss of genetic identity.

KEY WORDS   interspecific hybridization, biological control agent, ecological risk, nontarget effect, *Chrysoperla*

THE GREEN LACEWING, *Chrysoperla carnea* (Stephens), is frequently used for biological control. It has long been assumed to be a single morphologically identical species with a Holarctic distribution (Tjeder 1960). However, more recent evidence suggests that it is not a single species but instead a complex of several to many biological species characterized by different male courtship songs (Henry et al. 1993, 2001). In Japan, the indigenous green lacewing is widely distributed and has been categorized as *C. carnea* (Tsukaguchi 1985). However, it was revised to *C. nipponensis* (Okamoto) by Brooks (1994) based on external morphological differences such as the color of the gradate cross-veins, which are black in *C. nipponensis* and green in *C. carnea.* Its courtship song also differs from the other *carnea* group species (Henry and Wells 2004, Taki et al. 2005). In 1996, the green lacewing designated as *C. carnea* was imported from Germany on a test basis. It was registered as a biological pesticide in 2001 and is now on the market in Japan. Its gradate crossveins are primarily green.

The two species can now meet in the same habitat. Serious concerns over the nontarget impact of introduced exotic natural enemies on native ecosystems have been raised by a number of prominent ecologists and conservation biologists (Follett and Duan 2000, Wajnberg et al. 2001, Louda et al. 2003). Mochizuki and Mitsunaga (2004) showed that there were negligible nontarget impacts from interspecific predation between the introduced and the indigenous green lacewings. If they can hybridize readily, genetic modification of the indigenous *C. nipponensis* will occur, resulting in irreversible ecological risk, such as loss of genetic identity. Such genetic pollution is one of the potential nontarget impacts of introducing natural enemies.

Several cryptic biological species co-occur in Germany, including the true *C. carnea,* all of which are morphologically difficult to identify without courtship song analysis (Henry et al. 2002). They could have been imported in some shipments. In this paper, we treat the indigenous species as *C. nipponensis* and the introduced one as *C. carnea,* and study the hybrid compatibility among the introduced and indigenous green lacewings under laboratory conditions to identify any risk of destruction of the indigenous species' genetic integrity by the introduced exotic species.

[1] National Institute for Agro-Environmental Sciences; 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan.
[2] National Agricultural Research Center; 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan.
[3] Corresponding author, e-mail: lepi@affrc.go.jp.

Materials and Methods

Adult *C. nipponensis* were collected from NIAES (Tsukuba, Japan) fields. *C. carnea* larvae (Kagetaro) were purchased from Arysta LifeScience (Tokyo, Japan). The two species were reared in a similar manner. Approximately 50 females and 50 males were maintained in a 30 by 30 by 30-cm cage, supplied with water and a honey-yeast diet (a mixture of water, honey, and yeast extract as 10:10:3 mass ratio, respectively) and applied to absorbent cotton according to a modified version of the method of Henry (1979). Larvae were individually reared by supplying ≈20 mg of Entofood (frozen eggs of *Ephestia kuehniella;* Arysta LifeScience) every 2 d. Rearing and experiments were conducted under conditions of 16 L:8 D at 25°C.

Crosses were carried out with one pair of 7-≈14-d-old virgin adults, kept in a plastic cup (75 mm diameter by 45 mm height, 100 ml), supplied with water and a honey-yeast diet. There were 30 replicates each of parental conspecific crosses, *C. carnea* × *C. carnea* and *C. nipponensis* × *C. nipponensis*, and 63 replicates of interspecific crosses, *C. carnea* females × *C. nipponensis* males and *C. nipponensis* females × *C. carnea* males. Interspecific offspring (F1) were reared individually and supplied with Entofood, and emerged adults were crossed with each other or backcrossed to their parent species. We designed our F1 cross experiment to obtain an equal and maximal number of replicates using emerged F1 adults; consequently, we could ensure that all crosses of F1 adults were replicated by five pairs.

We examined the fertility, preoviposition period (defined as days from pairing to confirmation of the first fertile eggs), viability (hatchability and emergence rate), developmental period (from egg to adult), and sex ratio. Fertility was defined as the percentage of females that were fertile, i.e., that oviposited fertile eggs. Hatchability was calculated based on the number of eggs that hatched over the 3 d after the hatching of the first larva. For statistical analysis, we used 200 larvae randomly selected from all fertile pairs in conspecific crosses and >30 larvae from each interspecific and F1 cross.

We used the *G*-test to compare among the frequencies of hybridization (Sokal and Rohlf 1995). The data on fertility, preoviposition period, hatchability, emergence rate, developmental period, and sex ratio were analyzed by means of one-way analysis of variance (ANOVA); frequencies were arsine square-root transformed, and means were log-transformed to ensure the normality and homoscedasticity of our data. Frequencies and means were separated using the Tukey-Kramer test (Sokal and Rohlf 1995). The level of significance in all tests was 5%. The hatchability of (*C. carnea* female × *C. nipponensis* male) females × (*C. carnea* female × *C. nipponensis* male) males was removed from the statistical analysis, because only one of these pairs oviposited only two fertilized eggs during their lifespan, and no fertile eggs were confirmed during the 3-d observation period.

Results

Fertility differed significantly among crosses (*G*-test, df = 15, χ^2 = 1.3942; Table 1). Conspecific crosses within *C. carnea* or *C. nipponensis* showed high fertility (70.0% for *C. nipponensis*, 90.0% for *C. carnea*), but interspecific hybridization of *C. carnea* and *C. nipponensis* resulted in significantly lower fertility (41.3% for *C. carnea* females × *C. nipponensis* males, 9.5% for *C. nipponensis* females × *C. carnea* males). The fertility of crosses among F1 offspring was the same as for parental conspecific crosses, but crosses of F1 males derived from *C. carnea* females × *C. nipponensis* males produced no fertile eggs. Exceptionally, one pair of (*C. carnea* female × *C. nipponensis* male) females × (*C. carnea* female × *C. nipponensis* male) males produced 2 fertile eggs out of 242 eggs laid during the female's lifespan; however, 1 died as a second-instar larva and the other died at the pupal stage. Preoviposition period differed significantly between crosses (*F* = 4.10; df = 12,94; *P* < 0.0001; Table 1), with that of *C. nipponensis* females × *C. carnea* males being quite long (13.2 d), and the one fertile cross of (*C. carnea* female × *C. nipponensis* male) females × (*C. carnea* female × *C. nipponensis* male) males being 25.0 d.

Significant differences were found in the viabilities of each type of offspring (Table 2; hatchability: *F* = 2.46; df = 11,90; *P* = 0.0099, emergence rate: *F* = 2.73; df = 11,44; *P* = 0.0090). Hatchability was high in most crosses, but the offspring of (*C. nipponensis* female × *C. carnea* male) females × (*C. nipponensis* female × *C. carnea* male) males had by far the lowest hatchability (24.4%). Emergence rates of (*C. carnea* female × *C. nipponensis* male) females × (*C. nipponensis* female × *C. carnea* male) males and (*C. carnea* female × *C. nipponensis* male) females × *C. nipponensis* males were significantly lower (53.9% and 55.0%, respectively; Table 2).

Table 1. Fertility and preoviposition period of conspecific and interspecific crosses between *C. carnea* and *C. nipponensis*

Cross[a] ♀ ♂	Fertility (%)[b]	N	Preoviposition time (mean ± SD)[c]	N
n × n	70.0	30	3.3 ± 2.05 a	21
c × c	90.0	30	3.3 ± 1.84 a	27
c × n	41.3	63	4.4 ± 2.47 a	26
n × c	9.5	63	13.2 ± 9.33 bc	6
nc × nc	100.0	5	1.8 ± 0.50 a	5
nc × cn	0.0	5	—	0
cn × nc	80.0	5	2.0 ± 1.00 a	4
cn × cn	20.0	5	25.0 c	1
cc × nc	100.0	5	3.0 ± 2.71 a	5
nc × cc	80.0	5	5.0 ± 7.35 ab	4
nn × nc	80.0	5	4.5 ± 3.42 abc	4
nc × nn	100.0	5	2.8 ± 3.03 a	5
cc × cn	0.0	5	—	0
cn × cc	100.0	5	7.0 ± 4.80 abc	5
nn × cn	0.0	5	—	0
cn × nn	80.0	5	3.8 ± 4.86 ab	4

[a] n, *C. nipponensis;* c, *C. carnea;* e.g., nc represents the offspring of *C. nipponensis* females × *C. carnea* males.
[b] Fertility differed significantly among crosses (*G*-test).
[c] Values followed by a different letter are significantly different at the 5% level according to the Tukey-Kramer test.

Table 2. Hatchability and emergence rate of conspecific and interspecific crosses between *C. carnea* and *C. nipponensis*

Cross[a] ♀ × ♂	Hatchability (mean% ± SD)[b]	N	Emergence rate (mean% ± SD)[b]	N
n × n	71.6 ± 31.79 ab	21	66.7 ab	1
c × c	57.7 ± 39.13 ab	27	74.1 ab	1
c × n	78.7 ± 26.62 a	26	74.9 ± 27.89 a	26
n × c	87.5 ± 13.28 a	6	64.8 ± 27.42 ab	6
nc × nc	24.4 ± 15.35 b	5	58.8 ± 14.66 ab	5
nc × cn	—	0	—	0
cn × nc	51.5 ± 15.79 ab	4	53.9 ± 10.98 b	4
cn × cn	0.82	1	—	0
cc × nc	69.7 ± 13.16 ab	5	78.6 ± 11.73 ab	5
nc × cc	55.4 ± 11.48 ab	4	74.6 ± 5.54 ab	4
nn × nc	45.0 ± 26.51 ab	4	66.6 ± 5.07 ab	4
nc × nn	75.4 ± 11.43 ab	5	79.3 ± 10.58 ab	5
cc × cn	—	0	—	0
cn × cc	50.3 ± 6.62 ab	5	74.5 ± 11.11 ab	5
nn × cn	—	0	—	0
cn × nn	66.2 ± 10.86 ab	4	55.0 ± 15.36 b	4

[a] n, *C. nipponensis*; c, *C. carnea*; e.g., nc represents the offspring of *C. nipponensis* females × *C. carnea* males.
[b] Values within a column followed by a different letter are significantly different at the 5% level according to the Tukey-Kramer test.

Table 4. Sex ratio of conspecific and interspecific crosses between *C. carnea* and *C. nipponensis*

Cross[a] ♀ × ♂	N	Sex ratio (female proportion ± SD)[b]
n × n	1	0.50
c × c	1	0.49
c × n	9	0.56 ± 0.165
n × c	12	0.57 ± 0.111
nc × nc	5	0.53 ± 0.115
nc × cn	0	—
cn × nc	4	0.55 ± 0.094
cn × cn	0	—
cc × nc	5	0.49 ± 0.103
nc × cc	4	0.49 ± 0.065
nn × nc	4	0.56 ± 0.237
nc × nn	5	0.51 ± 0.026
cc × cn	0	—
cn × cc	4	0.49 ± 0.071
nn × cn	0	—
cn × nn	5	0.53 ± 0.070

[a] n, *C. nipponensis*; c, *C. carnea*; e.g., nc represents the offspring of *C. nipponensis* females × *C. carnea* males.
[b] No significant differences at the 5% level by ANOVA.

Developmental periods differed significantly by cross in both females and males (female: $F = 4.10$; df = 11,40; $P = 0.0004$, male: $F = 3.89$; df = 11,40; $P = 0.0007$; Table 3). In conspecific crosses, the male developmental period of *C. carnea* was significantly longer than in *C. nipponensis*, but that difference was not significant in females. Similar significant differences were found in interspecific crosses. The offspring of *C. nipponensis* females × *C. carnea* males required a longer developmental period than *C. carnea* females × *C. nipponensis* males. The offspring of (*C. nipponensis* female × *C. carnea* male) females × (*C. nipponensis* female × *C. carnea* male) males needed a significantly longer developmental period (male: 27.4 d, female:

Table 3. Developmental periods from egg to adult of conspecific and interspecific crosses between *C. carnea* and *C. nipponensis*

Cross[a] ♀ × ♂	N	Developmental period (mean ± SD)[b] Male	Female
n × n	1	21.3 ± 1.46 de	21.9 ± 1.63 cde
c × c	1	22.3 ± 1.65 bc	22.0 ± 1.52 cd
c × n	10	20.8 ± 2.43 ef	21.7 ± 3.10 cdef
n × c	8	22.8 ± 3.33 b	23.6 ± 3.43 b
nc × nc	2	20.9 ± 0.89 cdef	21.2 ± 0.69 cdef
nc × cn	0	—	—
cn × nc	5	27.4 ± 3.41 a	28.2 ± 2.48 a
cn × cn	0	—	—
cc × nc	4	21.9 ± 1.17 bcd	22.3 ± 1.59 c
nc × cc	4	21.2 ± 0.82 de	21.1 ± 0.76 ef
nn × nc	3	20.7 ± 0.73 ef	20.9 ± 0.89 ef
nc × nn	5	20.3 ± 1.07 f	20.8 ± 1.05 f
cc × cn	0	—	—
cn × cc	5	21.2 ± 0.91 de	21.2 ± 0.78 def
nn × cn	0	—	—
cn × nn	4	20.9 ± 1.34 def	21.6 ± 1.57 cdef

[a] n, *C. nipponensis*; c, *C. carnea*; e.g., nc represents the offspring of *C. nipponensis* females × *C. carnea* males.
[b] Values within a column followed by a different letter are significantly different at the 5% level according to the Tukey-Kramer test.

28.2 d) than did other crosses. The sex ratios of all crosses were similar at ≈1:1 (Table 4; $F = 0.42$; df = 11,44; $P < 0.9380$).

Discussion

In our experiment, the hybridization rate of the parental cross in the introduced green lacewing was 90%, and no hybrid breakdown was observed in successive generations. The courtship song of the offspring was the same as the true *C. carnea* (data not shown). In the indigenous green lacewing used here, hybridization rate among the parents was high (70%), and both the larval head capsule marking and the courtship song were the same as *C. nipponensis* (Henry and Wells 2004; *C. carnea* type A in Taki et al. 2005). Therefore, the population used in our experiment was thought to be the true *C. carnea* for the introduced species and *C. nipponensis* for the indigenous species, respectively.

When *C. carnea* and *C. nipponensis* were brought together in a small cup, they readily hybridized and produced fully viable and fertile F1 and F2 generations. However, interspecific hybridization resulted in low fertility (≤41.3%; Table 1). Albuquerque et al. (1996) showed similar low fertility in interspecific hybrids between *Chrysopa quadripunctata* and *C. slossonae* under laboratory conditions, although other studies of green lacewings, especially belonging to the carnea group, have reported highly fertile F1 and F2 hybrids and backcrosses between close relatives (*C. plorabunda* × *C. downesi*, Tauber and Tauber 1977; *C. plorabunda* × *C. johnsoni*, Wells 1993, Henry 1993, Henry et al. 1993). Under no-choice mating conditions, the barriers to prezygotic reproductive isolation seem to be broken in some insects (e.g., dos Santos et al. 2001). The low fertility of the interspecific hybrids in our experiment may result from postzygotic barriers, as in the case between *C. quadripunctata* and

C. slossonae, resulting from reproductive isolation having a negative effect on the sperm (Albuquerque et al. 1996). The absence of changes in hatchability, adult emergence rate, total developmental period, and sex ratio from the parents to the F2 generation of hybrids suggest that there is no genetic incompatibility between *C. carnea* and *C. nipponensis* that would cause hybrid breakdown. F1 males from *C. carnea* females × *C. nipponensis* males showed low fertility (close to 0%, but 20% in one case) when crossed with any female (F1 or backcross). In the case of the single pair that produced 20% fertile eggs, an extremely long preoviposition period was observed, which seemed to result from mating delay. However, the few eggs that hatched and developed to at least the second instar may be evidence of a degree of genetic compatibility.

In some orthopteran cryptic species, it has often been shown that courtship songs are the only differences that separate the species (Walker 1964). Comparable observations have been reported in several similar species, such as the ground crickets *Allonemobius faciatus* (De Geer) and *A. socius* (Scudder), and water bugs of the family Corixidae (Howard 1986, Jansson 1979). In closely related species, differences in courtship song seem to be the chief cause of the low success of hybridization. In green lacewings, Wells and Henry (1994) also showed that their courtship songs were an important barrier to interspecific hybridization. Notably, the courtship songs of the *carnea* group are quite elaborate and identically expressed in both sexes, and mating will not occur under natural conditions unless the participants engage in a prolonged and accurately matching duet (Wells and Henry 1992). Because the courtship songs of the introduced *C. carnea* and *C. nipponensis* are quite different (Henry et al. 2002, Henry and Wells 2004, Taki et al. 2005), interspecific mating between these two close relatives may not occur in the wild. There are no reports of natural hybrids between the two species or of naturalization of the introduced *C. carnea* in Japan. The problem of the cryptic biological species of the *carnea* group is complicated. In Europe, several biological species coexist (Henry et al. 2002), which could have been imported to Japan in some shipments. It is necessary to monitor how many biological species are imported from Germany labeled as *C. carnea* and their establishment in the wild, and study further their potential hybridization with the indigenous *C. nipponensis.*

Acknowledgments

References Cited

Albuquerque, G. S., C. A. Tauber, and M. J. Tauber. 1996. Postmating reproductive isolation between *Chrysopa quadripunctata* and *Chrysopa slossonae:* mechanisms and geographic variation. Evolution. 50: 1598–1606.

Brooks, S. J. 1994. A taxonomic review of the common green lacewing genus *Chrysoperla (Neuroptera: Chrysopidae).* Bull Br. Nat. Hist. Entomol. 63: 137–210.

dos Santos, P., K. Uramoto, and S. R. Matioli. 2001. Experimental hybridization among Anastrepha species (Diptera: Tephritidae): production and morphological characterization of F1 hybrids. Ann. Entomol. Soc. Am. 94: 717–725.

Follett, P. A., and J. J. Duan (eds.). 2000. Nontarget effects of biological control. Kluwer Academic Publishers, Boston, MA.

Henry, C. S. 1979. Acoustical communication during courtship and mating in the green lacewing *Chrysopa carnea* (Neuroptera: Chrysopidae). Ann. Entomol. Soc. Am. 72: 68–79.

Henry, C. S. 1993. *Chrysoperla johnsoni* Henry (Neuroptera: Chrysopidae): acoustic evidence for full species status. Ann. Entomol. Soc. Am. 86: 14–25.

Henry, C. S., and M.L.M. Wells. 2004. Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). Anim. Behav. 68: 879–895.

Henry, C. S., M. M. Wells, and R. J. Pupedis. 1993. Hidden taxonomic diversity within *Chrysoperla plorabunda* (Neuroptera: Chrysopidae): two new species based on courtship songs. Ann. Entomol. Soc. Am. 86: 1–13.

Henry, C. S., S. J. Brooks, D. Thierry, P. Duelli and J. B. Johnson. 2001. The common green lacewing (*Chrysoperla carnea* s. lat.) and the sibling species problem, pp. 29–42. *In* P. McEween and T. R. New (eds.), Lacewings in the crop environment. Cambridge University Press, Cambridge, MA.

Henry, C. S., S. J. Brooks, P. Duelli, and J. B. Johnson. 2002. Discovering the true *Chrysoperla carnea* (Stephens) (Insecta: Neuroptera: Chrysopidae) using song analysis, morphology, and ecology. Ann. Entomol. Soc. Am. 95: 172–191.

Howard, D. J. 1986. A zone of overlap and hybridization between two ground cricket species. Evolution. 40: 34–43.

Jansson, A. 1979. Postglacial distributional history of the water boatman *Arctocorisa carinata* Heteroptera Corixidae. Entomol. Gen. 6: 235–245.

Louda, S. M., R. W. Pemberton, M. T. Johnson, and P. A. Follett. 2003. Nontarget effects—the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annu. Rev. Entomol. 48: 365–396.

Mochizuki, A., and T. Mitsunaga. 2004. Non-target impact assessment of the introduced green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on the indigenous sibling species, *C nipponensis* (Okamoto) through interspecific predation. Appl. Entomol. Zool. 39: 217–220.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd ed. W. H. Freeman and Company. New York.

Taki, H., S. Kuroki, and M. Nomura. 2005. Taxonomic diversity within the Japanese green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), identified by courtship song analyses and crossing tests. J. Ethol. 23: 57–61.

Tauber, C. A., and M. J. Tauber. 1977. A genetic model for sympatric speciation through habitat diversification and seasonal isolation. Nature (Lond.). 268: 702–705.

Tjeder, B. 1960. Neuroptera from Newfoundland, Miqulon, and Labrador. Opuscula Entomol. 25: 146–149.

Tsukaguchi, S. 1985. A check list of published species of Japanese Chrysopidae (Neuroptera). Kontyû. 53: 503–506.

Wajnberg, E., J. K. Scott, and P. C. Quimby (eds.). 2001. Evaluating indirect ecological effects of biological control. CABI Publishing, Wallingford, UK.

Walker, T. J. 1964. Cryptic species among sound-producing Ensiferan Orthoptera (Gryllidae and Tettigoniidae). Q. Rev. Biol. 39: 345–355.

Wells, M. M. 1993. Laboratory hybridization in green lacewings (Neuroptera: Chrysopidae: *Chrysoperla*): evidence for genetic incompatibility. Can. J. Zool. 71: 233–237.

Wells, M. M., and C. S. Henry. 1992. The role of courtship songs in reproductive isolation among populations of green lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae). Evolution. 46: 31–42.

Wells, M. M., and C. S. Henry. 1994. Behavioral responses of hybrid lacewings (Neuroptera: Chrysopidae) to courtship songs. J. Insect Behav. 7: 649–662.