

Inbreeding Effects on *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae)

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ABSTRACT Up to 5 successive inbred generations were produced, through sibling mating, from 64 parental (P_0) pairs of *Propylea quatuordecimpunctata* (L.) collected from 3 locations in the northeastern United States. The percentage of P_0 females ovipositing >5 eggs was 62.5% compared with a mean of 43% for inbred generations F_1 to F_4 . The percentage of intercross (F_2 to F_4) females producing >5 eggs was $61 \pm 16\%$ (mean \pm SE), showing a restoration of fecundity. Egg hatch decreased from $84 \pm 9\%$ in the P_0 to $4 \pm 7\%$ in the F_4 . Preimaginal developmental time among inbreds increased from 17 d in the F_1 to >19 d in the F_4 . First instar to adult survivorship among inbreds decreased from $96.6 \pm 0.9\%$ in the F_1 to $37.6 \pm 3.7\%$ in the F_4 . In contrast to the inbreds, mean survivorship in the intercross generations was $85 \pm 11\%$, showing a restoration of viability. No effect of inbreeding on body size was detected. The high sensitivity to inbreeding in *P. quatuordecimpunctata* probably is caused by a high frequency of partially recessive deleterious alleles and overdominant loci.

KEY WORDS *Propylea quatuordecimpunctata*, inbreeding depression, body size, biological control, fitness traits, ladybeetles

Propylea quatuordecimpunctata (L.) is a Palearctic ladybeetle that is distributed widely in northeastern North America (Wheeler 1993, Day et al. 1994, Hoebeke and Wheeler 1996, Wheeler and Stoops 1996). The first records of *P. quatuordecimpunctata* in Québec in 1968 (Chantal 1972) suggest that adults were transported to North America on ships transiting the St. Lawrence Seaway, but additional colonizing events may have occurred (Wheeler 1993, Day et al. 1994, Yanega 1996).

Attempts in the 1960s to establish *P. quatuordecimpunctata* in North America failed (Rogers et al. 1972). More recently, no establishment has been observed from releases of >500,000 laboratory-reared *P. quatuordecimpunctata* adults in the central and western United States (Prokrym et al. 1993, Day et al. 1994). The failure to detect self-sustaining populations of *P. quatuordecimpunctata* is not unusual (Krafsur and Obrycki 1996); only 10% of the coccinellid species released in the United States have become established (Gordon 1985) compared with 34% obtained with all entomophagous species (Greathead 1986).

Genetic and ecological factors have been cited as important components influencing the establishment of natural enemies in new geographical locations (Messenger et al. 1976, Roush 1990, Hopper et al. 1993). The putative loss of genetic variation and its possibly harmful effects on field performance of mass-reared natural enemies has been discussed extensively (Mackauer 1976, Bartlett 1984, Joslyn 1984, Hopper et al. 1993, Roush and Hopper 1995). These discussions, however, were based on assumptions with no exper-

imental genetic data (Krafsur and Obrycki 1996). In ladybeetles, the loss of genetic variation by inbreeding also was presumed to be significant (Roush 1990). After 2 generations of sibling mating (Wright's inbreeding coefficient $F_w = 0.57$), the ladybeetle *Colomegilla maculata* (Degeer) showed a decrease of 37.5% in egg fertility, whereas no differences in fecundity and preimaginal development were detected (Kidd 1993).

Studies are required to determine if management of inbreeding is needed during laboratory rearing and to examine the heritability of traits that may be correlated with field performance of reared insects (Roush 1990, Ueno 1994). The objective of the current study was to determine the effects of inbreeding in *P. quatuordecimpunctata* on fecundity, fertility, sex ratio, developmental time, survivorship, and adult body size.

Materials and Methods

Source of Beetles. In July 1994, *P. quatuordecimpunctata* adults were collected in West Kingston, Washington County, RI, and in 2 locations in Jefferson County, NY. In June and July 1995, adults were collected from 2 locations in West Kingston, Washington County, RI, in Storrs, Tolland County, CT, and in Jefferson County, NY. Adults also were collected on 29 August 1996 in West Kingston, Washington County, RI. Geographical and genetic diversity of the *P. quatuordecimpunctata* founding stocks were substantial (Krafsur and Obrycki 1996).

Table 1. Percentage of inbred and intercrossed (*N*) *P. quatuordecimpunctata* females producing at least 5 eggs within 20 d

Year	Generation ^a				
	P ₀	F ₁	F ₂	F ₃	F ₄
1994	54.5 (33)	51.0 (102)	45.7 (70)	48.0 (25)	36.4 (11)
1995	66.7 (27)	29.2 (65)	41.7 (36)	50.0 (6)	—
1996	100 (4)	63.6 (11)	63.6 (11)	25.0 (4)	—
Mean	62.5	43.8	46.2	45.7	36.4
IC	—	50.0 (6)	53.8 (13)	80.0 (5)	—

^a Percentage of females producing at least 5 eggs within 20 d (number of females). IC, intercrossed generation in the laboratory.

Inbreeding. Beetles were reared at 22°C, 50% RH, and a photoperiod of 16:8 (L:D) h in 1994 and 1995; in 1996 rearing was at 24°C and 50% RH. In 1994, 33 pairs were set up (18 from New York and 15 from Rhode Island), 27 pairs were established in 1995 (7 from New York, 2 from Connecticut, and 18 from Rhode Island), and 4 pairs from Rhode Island were established in 1996. Sex was determined by using the pigmented anteromedian head of females (Rogers et al. 1971). Each pair was designated as a line, and all pairs from a collection site were designated as a population. Pairs were maintained separately for 20 d in 0.24-liter paper cages. Beetles were provided with water, a honey-Wheat (Qualcepts Nutrients, Minneapolis, MN) food mixture, and a daily supply of pea aphids, *Acyrtosiphon pisum* (Harris), and green peach aphids, *Myzus persicae* (Sulzer). Each pair was provided with a piece of white wax paper straw as an ovipositional substrate. Egg masses were collected and checked daily for hatch. Twenty to forty 1st instars from each line in each generation were reared. Eggs were then frozen to determine the eclosion rate for each line. First instars were transferred to individual glass vials, fed *A. pisum*, and checked daily for ecdysis.

Eggs from 1 of the 5 F₁ mating pairs from each line were randomly selected, and newly eclosed adults (F₂) were selected for rearing. Similar rearing procedures and data collection were followed for each successive generation until the F₅, by which time all lines had become extinct.

Measurements. Developmental time was recorded for each stadium. If death occurred, the date and stadium were recorded. Total survivorship was calculated as the percentage of 1st instars that successfully emerged from pupae. The sex of newly emerged adults (F₁) was determined, and up to 5 F₁ sibling breeding pairs per line were established. The remaining adults were frozen 24 h after eclosion.

Morphometric data collected included the body length (from tip of abdomen to tip of the head), elytra length (from the tip of abdomen to the mesothoracic suture), pronotum width, elytra width, and metatibia length.

Intercrosses. Because a large, randomly mating laboratory culture was not established as a control, crosses between inbred lines were made to determine if inbreeding effects observed were caused by genetic or environmental factors. An intercross consisted of an inbred male from one line mated with an inbred female from a different line. Selection of the intercross

adults was random. Six pairs were established from the F₁, 13 from the F₂, 5 from the F₃, and 1 from the F₄. From each intercross pair, up to forty 1st instars were reared in 1994, 20 in 1995, and 30 in 1996. Rearing procedures and data collection for intercross pairs and immatures were the same as those for inbred lines.

Data Analysis and Voucher Specimens. Developmental traits analyzed were developmental times and egg to adult viability. For analysis of developmental times, only data from 1994 and 1995 (temperature, 22°C) were included because 24°C was used in 1996.

Other fitness traits studied were fecundity, expressed as total eggs produced in 20 d, and fertility, expressed as the percentage of fertile eggs. Fecundity was estimated over the first 20 d because 55% of egg production occurred during that time (Morjan 1997). Females that laid <5 eggs were excluded because they were considered to be uniseminated or diapausing. The analysis included 9 populations. Studies done each year were considered replicates.

Data were analyzed using the general linear models procedure (PROC GLM, SAS Institute 1988). Means were compared using the Duncan test (SAS Institute 1988). Comparison between inbred and intercrosses was accomplished by using the Student *t*-test.

Because body size cannot be measured comprehensively in 1 measure, principal component analysis was used on the 5 variables measured. Morphometric variables were analyzed for each sex by using principal component analysis (PINCOMP, SAS Institute 1988). Body size was estimated by adding each morphometric variable multiplied by its eigen vector from the 1st principal component. The morphometric latent variable (size) was analyzed by using PROC GLM.

Voucher specimens are deposited in the Iowa State University Insect Collection, Ames.

Results

Proportion of Reproducing Females. Females (grouped by replicates, populations, and lines by generation) that produced at least 5 eggs decreased from 63% among beetles sampled from the field (P₀) to 36% for the F₄ (Table 1). From the F₁ to the F₃, the percentage of inbreds producing at least 5 eggs fluctuated between 44 and 47%. At least 5 eggs were produced by 50% of the F₁ intercross females, 53.8% of the F₂, and 80% of the F₃ (Table 1).

Fecundity. Field-collected beetles showed the highest percentage of females (27%) producing 100–

Table 2. Mean number of eggs produced in 20 d in inbred and intercrossed *P. quatuordecimpunctata*

Year	Population ^b	Generation ^a					
		P ₀	F ₁	F ₂	F ₃	F ₄	F ₅
1994	1	71.8 (6)	58.2 (10)	14.4 (5)	—	—	—
	2	104.4 (5)	25.8 (17)	53.5 (11)	83.5 (4)	5 (1)	—
	3	113.2 (5)	56.2 (20)	47.9 (10)	68 (8)	103 (3)	43.5 (4)
	4	49 (2)	35.1 (7)	37.3 (6)	—	—	—
1995	5	96.5 (2)	83 (2)	162 (1)	—	—	—
	6	113.1 (8)	78.5 (6)	63.2 (6)	7 (1)	—	—
	7	141.7 (4)	91.5 (6)	166.7 (3)	13 (1)	—	—
	8	70.2 (4)	173.6 (5)	147 (5)	167 (1)	—	—
1996	9	195 (4)	112.5 (8)	116 (7)	106 (1)	—	—
Mean		108.6	66.0	73.2	73.2	78.5	43.5
1994	IC	—	—	71 (4)	65 (4)	—	—
1995	IC	—	19 (1)	152 (1)	—	—	—
1996	IC	—	78 (2)	21 (2)	—	—	—
Mean	IC	—	58.3	68.3	65.0	—	—

^a Mean number of eggs produced in 20 d per female (number of females).

^b Population defined as beetles from a collection site. IC, intercrossed population in the laboratory.

200 eggs and females producing >200 eggs (8%). No females from the F₃ or F₄ produced >200 eggs, and most produced fewer eggs. No significant differences in fecundity were found among generations ($F = 0.93$, $df = 5$, $P = 0.48$) and populations ($F = 1.41$, $df = 6$, $P = 0.21$), but differences were observed among replicates ($F = 20.91$, $df = 2$, $P < 0.001$), the reason for which is obscure.

Outcrossed females produced 19–152 eggs (Table 2). Fecundity of intercross females was similar among generations ($F = 0.01$, $df = 2$, $P = 0.99$) and reps ($F = 0.10$, $df = 1$, $P = 0.91$). No differences in fecundity were observed between inbred and intercross generations ($t = 0.17$, $df = 5$, $P > 0.80$).

Fertility. The fertility of eggs in the P₀ (84%; weighted mean) was significantly greater than in the F₁ (50%). Fertility decreased to only 1% in the F₅. Fertility differences were significant among generations ($F = 75.35$, $df = 5$, $P < 0.001$), populations ($F = 3.83$, $df = 6$, $P = 0.001$), and reps ($F = 9.78$, $df = 2$, $P < 0.001$) (Fig. 1; Table 3).

Fertility of intercross matings averaged 59% (Table 3). The weighted mean fertility of the F₂ and the F₃ intercrosses was 55%, whereas the mean observed among the inbred F₂ and F₃ was 36%.

No differences in fertility of intercross females were found among generations ($F = 0.38$, $df = 2$, $P = 0.76$) and reps ($F = 1.27$, $df = 1$, $P = 0.34$).

Developmental Time. There was a progressive increase in developmental times with successive generations of inbreeding (Table 4). Differences were observed in larval developmental times among inbred beetles ($F = 14.05$, $df = 4$, $P = 0.001$) (Table 4). Examination of larval developmental times from a random sample of 105 larvae indicated that each instar had an incremental increase in developmental time with a higher level of inbreeding. Pupal developmental times also increased among inbred beetles ($F = 7.21$, $df = 4$, $P = 0.002$) (Table 4).

Viability. Declines in egg-to-adult survivorship (Table 5) were observed among successive inbred generations ($F = 87.18$, $df = 4$, $P < 0.001$) and populations

($F = 2.47$, $df = 6$, $P = 0.026$), but not among reps ($F = 0.01$, $df = 2$, $P = 0.99$). Problems in ecdysis seemed to correlate with mortality.

Intercross matings showed homogeneous viabilities of $85 \pm 11\%$ (mean \pm SE). No differences in survivorship were detected among intercross generations ($F = 0.1$, $df = 3$, $P = 0.91$) or reps ($F = 0.34$, $df = 2$, $P = 0.72$), so data were combined by generation; F₂ = 83.6%, F₃ = 87.5%, F₄ = 93.7%, and F₅ = 71.4%. Intercross individuals had greater survivorships than inbred individuals from the same generation ($t = 2.61$, $df = 5$, $P < 0.05$).

Body Size. The average body lengths ranged from 4.2 to 4.5 mm in females and 3.7 to 4.2 mm in males; body widths were 3.1–3.3 mm for females and 2.8–3.2 mm in males (Table 6).

Principal component analysis indicated that 1 principal component score could be used as a summary of the 5 original measurements because it accounted for $\approx 70\%$ of the total variance in body size. Coefficients for the 5 variables used to calculate the principal component were similar for both sexes (Table 7). Differences in body size were recorded among inbred generations ($F = 4.76$, $df = 5$, $P < 0.001$), populations ($F = 8.54$, $df = 6$, $P < 0.001$), and between sexes ($F = 639.43$, $df = 1$, $P < 0.001$), but replicates were homogeneous ($F = 2.74$, $df = 2$, $P = 0.06$). Within a generation, inbred females were on average 6% larger than males (Table 8).

Among intercrossed beetles, average body length ranged from 4.3 to 4.4 mm in females and 3.9 to 4.2 mm in males (Table 6). As in the inbred lines, the body length principal component accounted for $\approx 70\%$ of the total variance in body size and was used to describe adult size (Table 7). No differences in size were detected among intercross generations ($F = 0.59$, $df = 5$, $P = 0.66$). However, significant differences in body size of intercrosses were observed among reps ($F = 19.5$, $df = 2$, $P < 0.001$) and between sexes ($F = 17.1$, $df = 1$, $P < 0.001$). Intercross females averaged 3.4% larger than intercross males from the same generation. No significant differences in size ($t = 0.76$, $df = 5$, $P >$

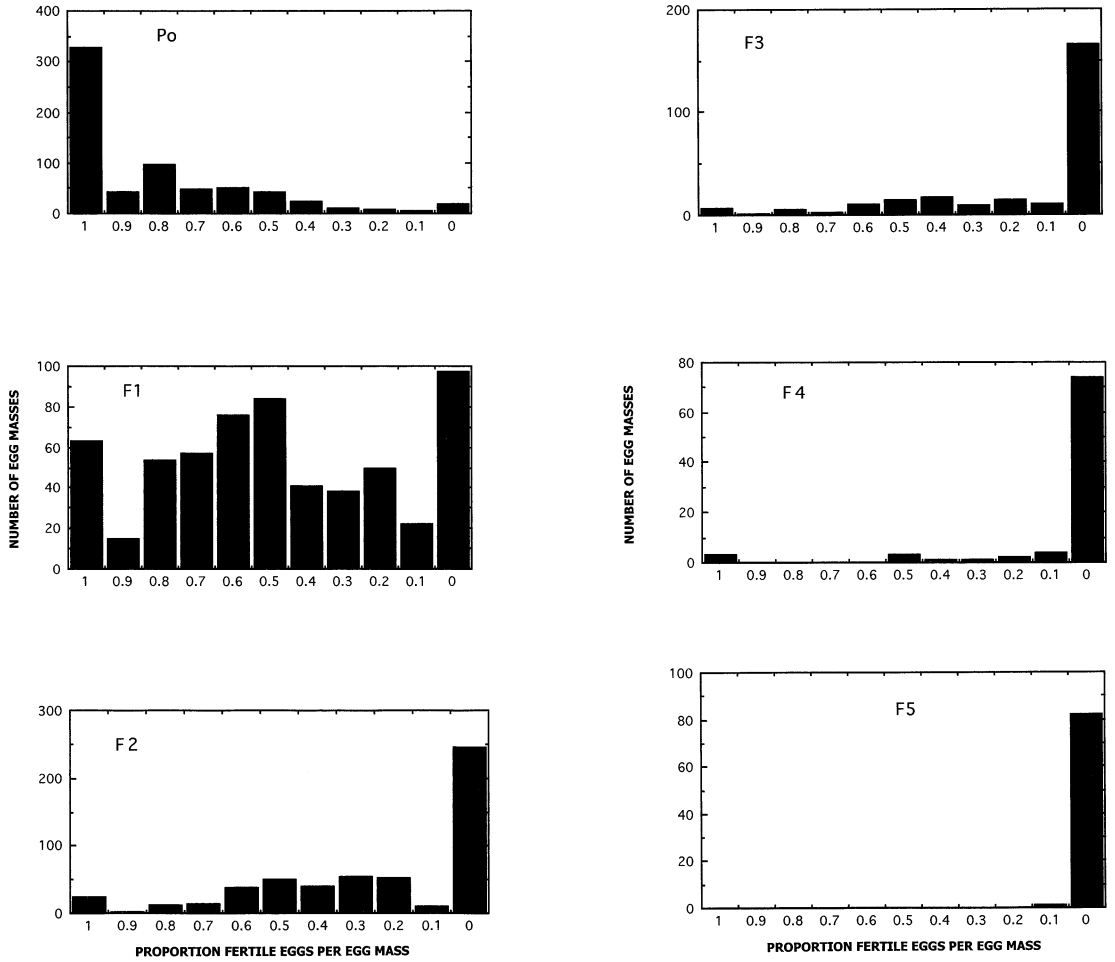


Fig. 1. Frequency distributions of the proportion of fertile eggs per egg mass for field-collected *P. quatuordecimpunctata* (P₀) and 5 inbred generations from 1994 and 1995. Number of egg masses recorded for each generation: P₀ (691); F₁ (602); F₂ (559); F₃ (185); F₄ (88); F₅ (83).

Table 3. Mean percentage of fertile eggs among inbred and intercrossed *P. quatuordecimpunctata*

Year	Population ^b	Generation ^a					
		P ₀	F ₁	F ₂	F ₃	F ₄	F ₅
1994	1	81 (6)	56 (10)	31 (5)	—	—	—
	2	82 (5)	43 (17)	19 (11)	20 (4)	0 (1)	—
	3	82 (5)	52 (20)	35 (10)	20 (8)	6 (3)	1 (4)
	4	94 (2)	46 (7)	33 (6)	—	—	—
1995	5	82 (2)	55 (2)	48 (1)	—	—	—
	6	84 (8)	44 (6)	11 (6)	0 (1)	—	—
	7	83 (4)	58 (6)	31 (3)	0 (1)	—	—
	8	94 (4)	66 (5)	30 (5)	10 (1)	—	—
1996	9	77 (4)	41 (8)	11 (7)	11 (1)	—	—
1994	IC	—	—	73 (4)	55 (4)	—	—
1995	IC	—	63 (1)	93 (1)	—	—	—
1996	IC	—	57 (2)	29 (2)	—	—	—

^a Mean percentage of fertile eggs produced in 20 d (number of females).

^b Population defined as beetles from a collection site. IC, intercrossed population in the laboratory.

Table 4. Egg to adult developmental time (mean \pm SD) by stadium in inbred and intercrossed *P. quatuordecimpunctata*

Year ^a	Generation (n) ^a	Developmental time, d			
		Egg	Larvae	Pupae	Total
1994	F ₁ (688)	3.4 \pm 0.5	9.5 \pm 1.0	4.2 \pm 0.6	17.2 \pm 1.2
	F ₂ (997)	3.8 \pm 0.6	10.5 \pm 1.7	4.5 \pm 0.6	18.5 \pm 1.6
	F ₃ (501)	4.0 \pm 0.6	11.9 \pm 2.7	4.6 \pm 0.6	19.6 \pm 1.7
	F ₄ (172)	4.1 \pm 0.8	11.8 \pm 2.0	4.9 \pm 0.7	20.6 \pm 2.0
	F ₅ (26)	4.0 \pm 0.7	12.4 \pm 2.0	5.2 \pm 1.0	20.1 \pm 1.1
1995	F ₁ (321)	3.2 \pm 0.6	9.6 \pm 0.9	4.1 \pm 0.5	17.0 \pm 1.0
	F ₂ (305)	3.5 \pm 0.7	10.4 \pm 1.4	4.3 \pm 0.6	18.2 \pm 1.1
	F ₃ (177)	3.8 \pm 0.7	11.0 \pm 1.2	4.4 \pm 0.6	18.9 \pm 1.3
	F ₄ (5)	4.2 \pm 0.7	10.3 \pm 1.2	5.0 \pm 1.0	19.0 \pm 1.0
IC 1994	F ₃ (128)	3.6 \pm 0.6	10.4 \pm 0.9	4.6 \pm 0.6	18.6 \pm 1.2
	F ₄ (95)	3.7 \pm 0.6	10.4 \pm 1.0	5.0 \pm 0.6	19.0 \pm 1.2
IC 1995	F ₂ (10)	2.4 \pm 0.5	9.6 \pm 0.7	4.0 \pm 0.0	16.0 \pm 0.8
	F ₃ (20)	4.4 \pm 0.5	10.6 \pm 0.7	4.5 \pm 0.6	19.5 \pm 0.8

^a IC, intercrossed generation in the laboratory.

0.4) were detected between intercross and inbred beetles (Table 8).

Mean Fitness. The foregoing data allow estimates of fitness of inbred *P. quatuordecimpunctata* relative to the outbred founding stocks. Fecundity, fertility, and viability were the chief fitness components affected by progressive inbreeding (Table 9). The relative proportions of ovipositing females, their mean fecundities, fertilities, and viabilities were multiplied to obtain fitness estimates (Table 10). The fitness of F₁ beetles averaged 25% of their outbred parental stocks and the succeeding generations averaged from 25 to 33% of their parents' fitnesses. Only a small proportion of F₄ beetles and no F₅ beetles succeeded in producing viable progeny (Table 9). The relationship between relative fitness, Y , and Wright's inbreeding coefficient, F_w , was found to be $Y = 0.002 - 0.09 \log_e F_w$ ($R^2 = 0.98$). The slope of this logarithmic function can be used to compare the relative fitness of species or strains in response to inbreeding.

Discussion

Effects of Inbreeding on *P. quatuordecimpunctata*. The negative effects of inbreeding on *P. quatuordecimpunctata* were greater than that recorded in 2 other

coccinellid species. In *P. quatuordecimpunctata*, a reduction of fertility from 84% in the P₀ to 50% in the F₁ indicated an inbreeding depression of 41%. This level of inbreeding depression was low compared with 61% depression observed in egg production in *Drosophila melanogaster* Meigen (Wright 1978). Reduced fertility in *P. quatuordecimpunctata* was partially recovered in the intercrosses (55% for the F₃), which demonstrates that inbreeding was a major factor affecting fertility. In the lady beetle, *C. maculata*, a 37.5% decline in fertility was observed after 2 consecutive sibling mating generations (Kidd 1993), much less than that observed in *P. quatuordecimpunctata*. In a 3rd lady beetle species, *Hippodamia variegata* Goeze, no significant loss in fertility was observed after 3 generations of sibling mating (unpublished data). No detectable loss of fertility was observed in the moth *Heliothis virescens* (F.) after 1 generation of sibling mating, even though fecundity decreased and immature mortality increased (Roush 1986). In a survey of insect and mammal species, inbreeding depression in fertility and fecundity ranged between 8 and 30% (Falconer 1989). Twelve to 17% inbreeding depression is typical in animals after 1 generation of sibling mating (Wright 1978). Fecundity was not affected by inbreeding in 2 pteromalid wasps, *Muscidifurax zaraptor* Kogan &

Table 5. Stage-specific percentage survival to adult stage of *P. quatuordecimpunctata* in successive generations of sibling matings

Year	Generation (n) ^a	Life stage					Total
		1	2	3	4	Pupae	
1994	F ₁ (688)	98.7	99.3	100	99.4	99.8	97.2
	F ₂ (997)	81.7	92.1	95.8	93.8	94.7	58.1
	F ₃ (501)	71.5	91.4	93.8	89.2	88.8	34.7
	F ₄ (172)	71.5	91.3	91.9	92.4	92.4	39.5
	F ₅ (26)	69.2	96.2	100	96.2	80.8	42.3
1995	F ₁ (321)	98.4	99.1	99.4	99.4	99.4	95.6
	F ₂ (305)	83.3	93.8	94.4	91.5	94.4	57.4
	F ₃ (177)	72.3	87.0	93.8	79.7	93.8	26.6
	F ₄ (5)	60.0	100	100	100	80.0	40.0
1996	F ₁ (120)	97.5	100	100	99.2	99.2	95.8
	F ₂ (167)	77.8	94.0	95.8	94.6	95.2	57.5
	F ₃ (66)	80.3	78.8	92.4	97.0	84.8	33.3
	F ₄ (12)	83.3	66.7	91.7	100	91.7	33.3

^a n, number of hatched eggs.

Table 6. Five morphometric measurements (mean ± SD) of inbred and intercrossed *P. quatuordecimpunctata* adults

Generation ^a	Sex (n)	Measurements, mm				
		Body length	Elytra length	Body width	Pronotum width	Tibia length
P ₀	♂♂ (14)	4.1 ± 0.15	3.0 ± 0.20	3.0 ± 0.16	2.1 ± 0.11	1.0 ± 0.08
	♀♀ (25)	4.4 ± 0.24	3.4 ± 0.23	3.2 ± 0.20	2.3 ± 0.13	1.0 ± 0.10
F ₁	♂♂ (438)	4.2 ± 0.26	3.2 ± 0.23	3.2 ± 0.17	2.3 ± 0.12	1.0 ± 0.10
	♀♀ (465)	4.5 ± 0.23	3.5 ± 0.21	3.3 ± 0.16	2.4 ± 0.13	1.1 ± 0.09
F ₂	♂♂ (320)	4.1 ± 0.29	3.1 ± 0.26	3.1 ± 0.19	2.2 ± 0.12	1.0 ± 0.11
	♀♀ (318)	4.4 ± 0.34	3.4 ± 0.28	3.2 ± 0.26	2.3 ± 0.15	1.1 ± 0.13
F ₃	♂♂ (135)	3.9 ± 0.45	3.0 ± 0.27	3.1 ± 0.23	2.2 ± 0.14	1.0 ± 0.11
	♀♀ (109)	4.3 ± 0.39	3.3 ± 0.28	3.2 ± 0.23	2.3 ± 0.21	1.0 ± 0.13
F ₄	♂♂ (35)	3.8 ± 0.22	3.0 ± 0.25	2.9 ± 0.26	2.2 ± 0.12	1.0 ± 0.10
	♀♀ (23)	4.2 ± 0.27	3.4 ± 0.20	3.2 ± 0.19	2.3 ± 0.06	1.1 ± 0.09
F ₅	♂♂ (4)	3.7 ± 0.17	2.8 ± 0.07	2.8 ± 0.11	2.4 ± 0.13	1.1 ± 0.06
	♀♀ (4)	4.2 ± 0.13	3.3 ± 0.27	3.1 ± 0.06	2.3 ± 0.06	1.1 ± 0.12
IC F ₁	♂♂ (8)	4.2 ± 0.48	3.2 ± 0.3	3.1 ± 0.31	2.2 ± 0.17	1.0 ± 0.13
	♀♀ (12)	4.4 ± 0.27	3.4 ± 0.27	3.3 ± 0.18	2.3 ± 0.14	1.1 ± 0.09
IC F ₂	♂♂ (41)	3.9 ± 0.22	3.0 ± 0.23	3.1 ± 0.20	2.2 ± 0.16	1.0 ± 0.11
	♀♀ (23)	4.3 ± 0.15	3.4 ± 0.24	3.3 ± 0.19	2.3 ± 0.12	1.1 ± 0.08
IC F ₃	♂♂ (87)	4.0 ± 0.24	3.0 ± 0.20	3.1 ± 0.15	2.2 ± 0.09	1.0 ± 0.06
	♀♀ (61)	4.4 ± 0.17	3.3 ± 0.25	3.3 ± 0.21	2.3 ± 0.09	1.1 ± 0.07
IC F ₄	♂♂ (49)	3.9 ± 0.18	3.0 ± 0.22	3.0 ± 0.17	2.2 ± 0.11	1.0 ± 0.07
	♀♀ (40)	4.3 ± 0.19	3.3 ± 0.22	3.3 ± 0.14	2.3 ± 0.11	1.1 ± 0.08

^a P₀, parental; IC, intercrossed population in the laboratory.

Legner and *M. raptor* Girault & Sanders (Legner 1979), and the phytoseiid mite *Metaseiulus occidentalis* (Netsbitt) after 9 generations of sibling mating (Hoy 1977). In these haplodiploid species, deleterious alleles are screened out in males.

The low levels of survivorship observed in early instars of inbred *P. quatuordecimpunctata* support the hypothesis that lethal and semilethal recessive alleles were present in high frequencies in the founding stocks (Schields 1993, Lynch and Walsh 1998). In *P. quatuordecimpunctata*, an average decrease of 37% was observed in larval survivorship after only 1 round of sibling mating. In contrast, larval survivorship in *H. variegata* decreased by only 10% after a round of sibling mating (unpublished data). Similarly low levels of inbreeding depression were observed in larval mortality in *H. virescens* after 1 generation of sibling mating (Roush 1986).

Developmental times of inbred *P. quatuordecimpunctata* increased with successive rounds of brother-sister matings. In contrast, in *H. variegata* (unpublished data) and *C. maculata* (Kidd 1993), the chrysomelid beetle, *Galerucella calmeriensis* L. (Kidd 1993), and the moth, *H. virescens* (Roush 1986), larval developmental time was not affected by inbreeding. Growth rate in fishes, amphibians, and reptiles has

been observed to decrease because of inbreeding (Waldman and McKinnon 1993).

The morphometric data indicated that body size in *P. quatuordecimpunctata* was largely unresponsive to inbreeding and therefore demonstrated high levels of additive genetic variance, a typical result. High levels of additive genetic variance do not apply to characters strongly related to fitness because selection optimizes fitness (Falconer 1989, Lynch and Walsh 1998). Reductions of <5% in the thorax and wing length were observed in lines of *D. melanogaster* with an inbreeding coefficient *F_w* = 0.8 (Robertson 1954, Wright 1978).

The close inbreeding in this study gave levels of inbreeding coefficients (*F_w*) of 0.25 in the 1st generation, 0.44 in the 2nd, 0.58 in the 3rd, 0.68 in the 4th, and 0.76 in the 5th. Few lines survived until the 4th round of inbreeding, and 1 line of F₅ reached adulthood. Only 4 of 11 F₅ females oviposited, and 2 of their 174 eggs hatched. Crosses between any 2 inbred *P. quatuordecimpunctata* lines, however, restored much of the lost fitness, indicating that several loci were involved. It therefore appears that *P. quatuordecimpunctata* possesses a high frequency of recessive lethal and subvital mutations. In principle, this "genetic load"

Table 7. Principal component coefficients of inbred and intercrossed *P. quatuordecimpunctata* for body parts

Variable ^a	Inbred		Intercrossed	
	♀♀	♂♂	♀♀	♂♂
BLENGTH	0.69	0.73	0.76	0.67
ELENGTH	0.49	0.49	0.44	0.54
BWIDTH	0.42	0.39	0.38	0.42
PWIDTH	0.27	0.23	0.25	0.26
TLENGTH	0.16	0.14	0.13	0.13

^a BLENGTH, body length; ELENGTH, elytra length; BWIDTH, body width; PWIDTH, pronotum width; TLENGTH, metatibia length.

Table 8. Average size (morphometric score) of inbred and intercross (IC) generations of *P. quatuordecimpunctata*

Generation ^a	1994		1995		1996	
	Mean (<i>n</i>)	SD	Mean (<i>n</i>)	SD	Mean (<i>n</i>)	SD
P ₀	51.8 (4)	1.80	50.9 (29)	3.00	53.9 (6)	2.60
F ₁	52.0 (501)	2.84	53.3 (300)	2.91	51.9 (100)	3.55
F ₂	50.7 (399)	3.65	52.2 (160)	3.31	50.4 (77)	3.25
F ₃	49.7 (182)	3.85	50.3 (46)	4.90	47.8 (16)	4.15
F ₄	48.4 (56)	3.10	54.5 (2)	1.85	—	—
F ₅	48.0 (8)	3.13	—	—	—	—
IC F ₁	43.6 (3)	2.75	52.1 (9)	2.13	51.5 (8)	2.51
IC F ₂	49.0 (18)	3.15	49.9 (15)	1.67	48.4 (31)	2.39
IC F ₃	48.9 (120)	2.04	50.0 (16)	2.14	50.8 (12)	2.55
IC F ₄	48.9 (89)	2.28	—	—	—	—

Mean morphometric score equals the sum of each measurement (body, elytra, tibia length, elytra, body width) x its principal component coefficient (from Table 7). Data for each year are based on individuals from all families of all populations. Scale: 7.75 units = 1 mm. (*n*), number of adults.

^a IC, intercrossed generation in the laboratory.

(Wallace 1970) could be caused by a balanced system where homozygotes at 1 or more loci were lethal, subvital, or in some way or combination less fit than the heterozygotes.

Mean Fitness. Our data show that inbreeding caused a severe loss of fitness in *P. quatuordecimpunctata*. Brother-sister matings among the progeny of outbred beetles were less likely to oviposit and those that did so were less fecund (Table 9). Moreover, there was an immediate loss of fertility, and eggs that hatched showed reduced viabilities and longer developmental times. The overall fitness of the F₁ was estimated to be only 25% of the outbred parents, ignoring the slower developmental times (Table 10). Each succeeding generation showed a similar percentage loss of fitness. This is an unexpectedly large effect of inbreeding and raises the question of its causes.

Origin of Genetic Load in *P. quatuordecimpunctata*. It is easy to assume that the high frequency of deleterious genes in *P. quatuordecimpunctata* was caused by inbreeding that correlated with its establishment in North America. However, allozyme variation of 33 alleles segregating at 11 loci has shown a mean gene diversity of $15.2 \pm 2.5\%$ in 4 New World populations and $11.8 \pm 4.9\%$ in 5 Old World populations, 4 of which were in culture. These diversities were homogeneous ($t = 1.28$, $df = 7$, $P = 0.24$ [Krafsur and Obrycki 1996]), leading to the conclusion that New World *P. quatuordecimpunctata* was not detectably inbred with re-

spect to Old World beetles. Therefore, it seems very likely that the putative genetic load uncovered by close inbreeding of naturalized *P. quatuordecimpunctata* is characteristic of the species. The level of inbreeding depression in *P. quatuordecimpunctata* seems extraordinary. Are there high frequencies of deleterious recessive alleles segregating in *P. quatuordecimpunctata*, or are there overdominant loci, or both? It is commonly assumed that natural selection would keep the frequency of deleterious alleles quite low unless the mutation rates were unusually high (Lynch and Walsh 1998). Balanced polymorphisms maintained at overdominant loci by heterozygote superiority provide an alternative explanation. Indeed, inbreeding depression is a consequence of dominance and overdominance (Lynch and Walsh 1998). It seems unlikely that there is a locus that mutates at such a rate as to maintain a recessive lethal at a frequency of ≈ 0.85 in the face of strong selection against homozygotes. But several recessive and partially recessive loci may be segregating. Restoration of fitness among progeny of intercrossed inbred lines supports the view that recessive, deleterious alleles caused the inbreeding depression. Moreover, the overdominance hypothesis would seem to predict a fitness asymptote well above zero, but the inbreeding data showed that fitness declined almost linearly to zero.

It is unlikely that the severe levels of inbreeding obtained in the current study would occur among

Table 9. Fecundity, fertility, and viability in parental *P. quatuordecimpunctata* and 5 inbred generations

Generation	<i>n</i> ^a	Inbreeding coefficient ^b	Mean fecundity	Mean fertility \pm SD ^c	% decrease in fertility between successive generations
P ₀	40	0	108.6	0.84 \pm 0.09a	0
F ₁	81	0.25	66.0	0.50 \pm 0.20b	40.7
F ₂	54	0.37	73.2	0.25 \pm 0.21c	49.7
F ₃	16	0.50	73.2	0.16 \pm 0.19cd	35.5
F ₄	4	0.59	78.5	0.04 \pm 0.07d	73.3
F ₅	4	0.67	43.5	0.005 \pm 0.01d	88.2

^a *n*, number of females.

^b Theoretical proportion of homozygous loci relative to founding stock.

^c Means followed by the same letter are not statistically different (Duncan test).

Table 10. Fitness components of inbred *P. quatuordecimpunctata* relative to outbred, parental founding stock

Generation	Inbreeding coefficient ^a	% ovipositing	Relative			
			Fecundity	Fertility	Viability	Fitness
P ₀	0	1.0	1.0	1.0	1.0	1.0
F ₁	0.25	0.70	0.61	0.59	0.96	0.24
F ₂	0.37	0.74	0.67	0.30	0.58	0.09
F ₃	0.50	0.73	0.67	0.19	0.33	0.03
F ₄	0.59	0.58	0.72	0.05	0.34	0.007
F ₅	0.67	—	0.40	0.006	0	0

^a Theoretical proportion of homozygous loci relative to founding stock.

natural populations, but brother–sister matings among the progeny of outbred parents is probably not uncommon in this colonizing species. Most ladybeetles are colonizing species; in many instances, a single inseminated female may discover a patch of prey and begin to reproduce. In such situations, her progeny would probably undergo a round of sibling mating unless phenological or other mechanisms prevented it. A comparative study of sexual maturation, mating behavior, and dispersal characteristics in and among ladybeetle species is warranted.

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