

Evolutionary Character Changes and Population Responses in an Insect Host-Parasitoid Experimental System

Midori TUDA¹⁾

Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Abstract. In an insect host (the cowpea weevil *Callosobruchus maculatus*)-parasitoid (*Heterospilus prosopidis*) experimental system, the population densities of the component species oscillated for the first 20 generations and then abruptly stabilized as the parasitoid density decreased. Examination of the host and parasitoid after the 40th generation in the long-term experiment showed that (1) host larvae exhibited contest-type competition (killing other larvae inhabiting the same bean), in contrast to the founder population being scramble-type competitors and (2) the parasitoid attack rate on the host did not change. There was also an evolutionary trade-off between body size and the rates of larval survival and development, suggesting a cost of contest competition on larval survivorship and development. I tested model predictions (Tuda and Iwasa 1998) that (1) host equilibrium population size should gradually decrease as the proportion of the contest type increases and that (2) random attacks of the parasitoid on the host should reduce the rate of increase in proportion of the contest type, and the effect should become manifest especially during the first 20 generations. Two of three host-only replicates showed significant decrease in population sizes. Although the density of emerging adults per bean did not differ between replicates of the host-only and host-parasitoid systems, comparison of the host body size between them on day 270 (at the 13th generation) showed that the host was more contest-type in the host-only system than in the host-parasitoid system, as the model predicted, and later on day 650 the effect of the parasitoid had disappeared.

Key words: *Callosobruchus maculatus*, contest competition, evolutionary dynamics, *Heterospilus prosopidis*, host-parasitoid system.

Introduction

Observations on temporal changes in phenotypic characters as a consequence of biological interactions have been accumulated as direct evidence of evolution (e.g. Nicholson 1957; Stearns 1983; Brakefield 1987; Stokes et al. 1988; Reznick et al. 1990). Such evolutionary changes in characters, however, have rarely been recognized as the driving force of long-term changes in population dynamics of interacting species. For species populations, life-history evolution has been well studied both empirically and theoretically (Roff 1992; Stearns 1992). Yet, life-history evolution of two or more interacting species has hardly been studied, especially for host-parasitoid or prey-predator systems.

In an experimental host (the cowpea bean weevil *Callosobruchus maculatus*)-parasitoid (a braconid wasp

Heterospilus prosopidis) system, population densities of the component species oscillated for the first 20 generations and then abruptly stabilized as the parasitoid density decreased (Tuda and Iwasa 1998). Change of fluctuations and average levels in the population dynamics were ascribed to character evolution in the type of host larval competition. A theoretical study (Tuda and Iwasa 1998) showed that the contest type (killing other larvae inhabiting the same bean) was evolutionarily stable under the experimental conditions, and the mean proportion of the contest type increased rapidly within 20 generations. The scramble type stayed at the peripheral part of beans (the resource for the host), while the contest type dominated at the center of the beans. This model showed that the size of the bean used as a resource for the host when sufficiently small forced the scramble-type larvae to frequently encounter the contest-type larvae and to be killed.

The present study examined evolutionary changes in the host and parasitoid during 800 days or 40 host generations

¹⁾ E-mail: tuda@gri.kyushu-u.ac.jp

in a host-parasitoid system and the correlation between life history characters. Furthermore, I tested model predictions (Tuda and Iwasa 1998) that (1) the host equilibrium population size should gradually decrease as the proportion of the contest type increases and that (2) random attacks of parasitoid on the host should reduce the rate of increase in proportion of the contest-type hosts for the initial 20 generations, after which the proportion should converge to that in the host-only population.

The host-parasitoid system

Preparation and maintenance of the host-parasitoid experimental system is described in Tuda (1996). Briefly, an experimental arena (W120×D120×H30 mm) consisted of four mung bean (*Vigna radiata*; 48.4±6.4 mg, mean±SD, $n=20$) patches, which served as a resource for the bean weevil *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Five grams of beans were placed every 10 days into one of the four patches, each of which was replaced with the same amount of fresh beans every 40 days. Ten pairs of bean weevils were introduced on days 0, 10 and 20, and the braconid wasp *Heterospilus prosopidis* Vier. (Hymenoptera: Braconidae) was introduced on day 60. During resource replacement, the numbers of live and dead adults were recorded every 10 days and dead ones removed. The number of adults which emerged were calculated as (no. of live adults)+(no. of dead adults) — (no. of live adults 10 days previously). The experimental system was set up in August, 1993. All experiments were performed under laboratory conditions, at 30°C, 70% and 16L:8D. The host population had been maintained on azuki beans *Vigna angularis* (148.8±27.5 mg, mean±SD, $n=20$) in a laboratory at the University of Tokyo since 1992 (M. Shimada personal communication). The parasitoid population had been kept on *C. chinensis* at several laboratories since the early 1940's (S. Utida, K. Fujii, personal communication). To quantify stability, the dominant Lyapunov exponents, whose negative absolute values indicate the largest exponential rate of convergence between trajectories with infinitesimally small initial difference, were estimated by LENNS (Ellner et al. 1992).

Change in the property of population dynamics was observed around day 410 and the time series was divided into two census periods (days 60–410 and days 420–800). The population level was lower in the latter census period (10.0±8.0; mean±SD) than in the former (73.9±50.8) for the parasitoid ($t=7.76$, $df=73$, $P<0.001$), while it was higher in the host (the latter 73.4±14.7 compared to the former 55.1±34.6; $t=3.03$, $df=73$, $P<0.001$). The dominant Lyapunov exponents of the population dynamics were all negative and their absolute values were larger

(i.e. faster convergence of two close trajectories) in the later census period both in the host and parasitoid (host, the former, -5.51 ± 0.04 ; mean±SE, the latter, -5.86 ± 0.02 ; parasitoid, the former, -0.07 ± 0.00 , the latter, -4.52 ± 0.02), indicating more stability in the latter period.

Detection of character changes in host-parasitoid system

Experiments were done during late 1995 to early 1996, after sampling insects from the host-parasitoid system on the 810th day. Hereafter the progeny of the sampled host and parasitoid will be referred to as **HP** populations compared to **HO** (host-only) population. Stock culture populations of host and parasitoid as founders of the system will be referred to as **S** populations.

Emergence per bean and life history characters of the host

Host adults were allowed to oviposit on mung beans (50–60 mg) for 24 h. Four days after the oviposition, egg densities per bean were adjusted to one, two, three, four and five, and 20 beans of each egg density were placed in of plastic box wells (Corning: 96 wells). Fresh weights of emerging adults grown singly in beans were measured daily and the number of emerging adults from beans was recorded five weeks after oviposition. To determine the numbers of adult emergence in the stock population I first compared two data sets each collected in 1994 and in 1995 to ensure that there were no changes in the maximum emergence per bean, and used the 1994 data for comparisons. The developmental period of hosts grown singly was examined by recording adults emerging from 50 beans daily. To calculate survival rate, another set of 50 beans with a single developing larva was prepared and emerging adults were counted five weeks after egg deposition. A two-way ANOVA was applied, unless otherwise noted in the following or in the above text. Mann-Whitney *U* tests were used on persistence times and fecundity, and *t*-tests were applied to population levels and the density of emerging adults in the stock host population to detect any long-term changes. Linear regression was used to test for a decreasing trend in the population size with time in the host-only system. A nonparametric two-way ANOVA was applied to data on developmental periods and longevity (Zar 1984). *G*-tests were used to compare survival rates.

Density of emerging host adults per bean was lower in **HP**₁ than in **S** ($F=49.1$, $df=1$, $P<0.001$, Fig. 1) and the difference was due to density dependence acting on hatched larvae ($F=10.2$, $df=4$, $P<0.001$ for hatched larvae density; $F=3.26$, $df=4$, $P<0.05$ for interaction).

The maximum density of emerging adults at four hatched larvae per bean was not different between 1994 and 1995 (the former, 2.42 ± 0.23 , $t=0.240$, $df=22$, $P>0.5$, Fig. 1).

The body weight of bean weevil adults was greater in **HP₁** than in **S** ($F=75.4$, $df=1$, $P<0.001$, Fig. 2). A difference between sexes was also detected ($F=263$, $df=1$, $P<0.001$) but none for the interaction effect between population and sex ($F=3.02$, $df=1$, $P>0.05$).

Other life history characters are shown in Table 1: Fecundity was not different between **S** and **HP₁** ($U_{20,20}=206.5$, $P>0.5$). Longevity of adults was greater in the **HP₁** population than in the **S** population and no difference between sexes or interaction effect were detected ($H=5.29$, $P<0.05$ for **S** and **HP₁** populations; $H=2.97$, $P>0.05$ for sex; $H=0.311$, $P>0.5$ for interaction). The developmental period of bean weevils grown singly was longer in **HP₁** than in **S** ($H=9.68$, $P<0.005$). No effects

of sex and its interaction with populations were present ($H=0.00811$, $P>0.5$ for sex; $H=1.56$, $P>0.1$ for interaction). Survival rate of larvae grown singly was lower in **HP₁** than in **S** ($G=6.43$, $P<0.05$).

Attack rate of the parasitoid

I examined a possible alteration of the host-parasitoid interaction resulting from the development of host resistance and parasitoid behavioral modification, which was not assumed in the model by Tuda and Iwasa (1998). Two parasitoid females which emerged within 24 h were allowed to be inseminated for a day. They were provided with 25 mung beans each containing a 14-day-old host larva for 24 h. The two parasitoid populations **S** and **HP₁** were used in combination with the two different host populations, **S** and **HP₁**. After 18 days, the number of emerging parasitoid adults was checked. A two-way ANOVA was used to test for differences in attack rates.

There was no difference between host populations or

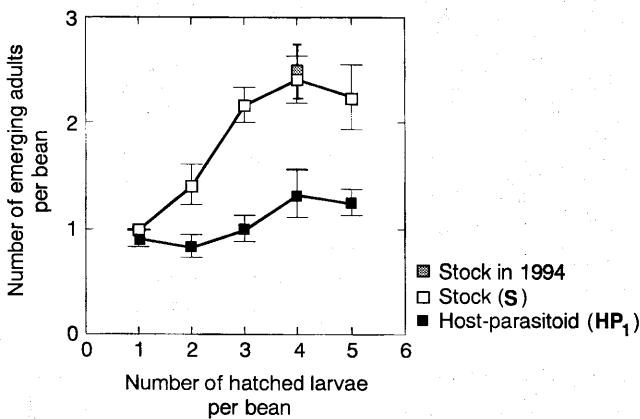


Fig. 1. Density of emerging adults grown at different larval densities per bean (mean ± SE). Open squares show the stock population of the bean weevil (**S**) and solid squares show bean weevils sampled from the host (bean weevil)-parasitoid system on day 810 (**HP₁**). A hatched square shows the stock population a year after.

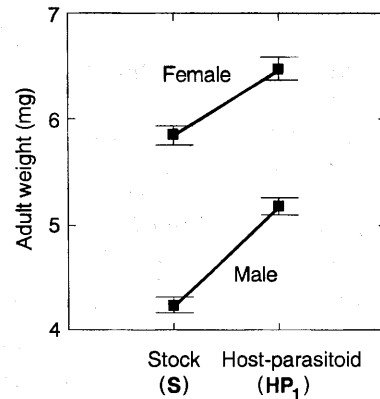


Fig. 2. Fresh weights (mean ± SE) of emerging adults of the stock population (**S**) and the host-parasitoid system (**HP₁**). Sample sizes were 10 for each combination of sex · population.

Table 1. Life history changes of the host in the host-parasitoid system (**HP₁**) compared to the stock population (**S**).

Life history character	S population		HP ₁ population		
	Mean ± SE	n	Mean ± SE	n	
Larval survival rate	0.96	100	0.86	100	*
Developmental period (days)					**
Female	22.42 ± 0.36	24	24.23 ± 0.51	22	
Male	22.67 ± 0.30	24	24.00 ± 0.88	22	
Fecundity	83.00 ± 1.72	20	79.05 ± 5.04	20	N
Adult longevity (days)					*
Female	7.15 ± 0.22	20	7.80 ± 0.36	20	
Male	7.80 ± 0.27	20	8.20 ± 0.22	20	

N: non-significant, *: $P<0.05$, **: $P<0.005$

between parasitoid populations in the attack rate of the parasitoid ($F=0.758$, $df=1$, $P>0.1$ for the parasitoid populations; $F=0.337$, $df=1$, $P>0.5$ for the host populations; $F=3.03$, $df=1$, $P>0.1$ for the interaction, Fig. 3).

To summarize, in the host-parasitoid system which persisted for more than 800 days or 40 host generations, the resultant host's characters had changed; i.e. developmental period, larval survival rate, adult longevity, adult weight and the maximum density of emerging adults, the last two of which especially indicate a shift in larval competition from scramble to contest type. By contrast, the attack rate of the parasitoid was not affected by the interaction between the host and parasitoid.

Test on effect of parasitization on host evolutionary speed

Model prediction

A model (Tuda and Iwasa 1998), incorporating estimated parameter values for the contest- and scramble types, predicts that random attacks by a parasitoid should slow evolutionary change in the host larval competition to the contest type, and that the effect should become manifest especially during the first 20 generations or 400 days. Also predicted is a gradual decrease in host population size in a parasitoid-free, host-only system, as the contest type dominates the population. In this section these predictions will be tested experimentally.

Host-parasitoid and host-only systems

Host-parasitoid system (**HP₂**) and host-only systems (**HO**) were set up to test the predicted difference in the speed of evolution between the two systems. Three replicates of

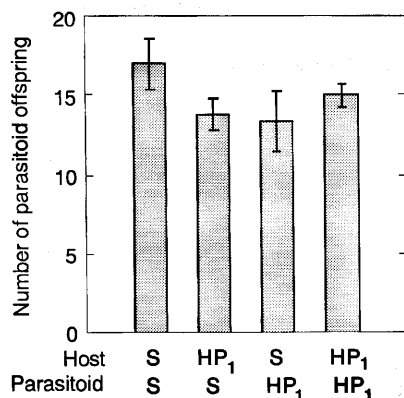


Fig. 3. Attack rates (mean \pm SE) of the two parasitoid populations, stock (**S_p**) and host-parasitoid system (**HP₁**), on the two host populations, stock (**S_H**) and host-parasitoid system (**HP₁**).

each system were set up as described previously, except no parasitoids were introduced in the **HO** system.

Persistence times of the host-parasitoid system, or the time to extinction of either species, were 300, 330 and ≥ 670 days (indicated by arrows in Fig. 4a), and there was no difference between the system started in 1993 and the present system ($U_{3,4}=6.0$, $P=1.0$, Fig. 4a). There was a decreasing trend in the population sizes of the host after an initial transient phase (i.e. day $60 \leq$) in two of the three replicates of the host-only system (Fig. 4b, replicate 1, $t=2.57$, $df=39$, $P<0.05$; Fig. 4c, replicate 2, $t=1.54$, $df=$

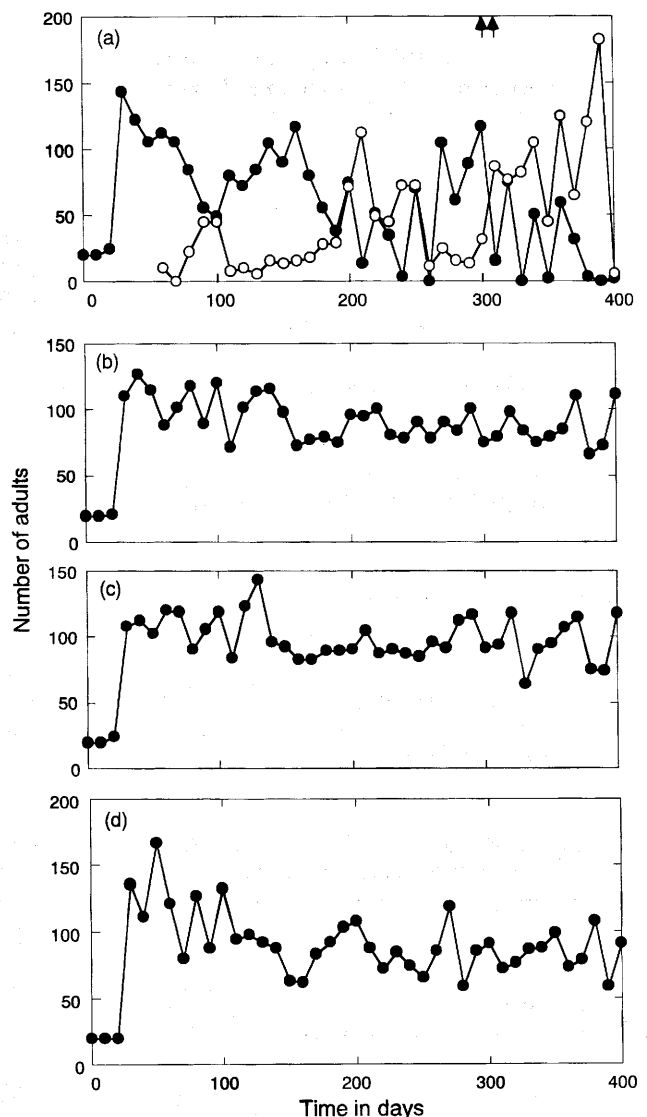


Fig. 4. (a) A representative replicate of the host-parasitoid system dynamics and (b)-(d) all replicated dynamics of the host-only system. Solid circles show host population sizes and open circles show parasitoid population sizes. Arrows in (a) indicate census dates when extinction of either component species was confirmed.

39, $P > 0.1$; Fig. 4d, replicate 3, $t = 2.19$, $df = 37$, $P < 0.05$).

Emergence per bean and weight of host adults

As an index of the degree of contest competition of larval competition in the host, I used adult weight and maximum saturating density of emerging adults per bean, the latter of which includes the original definition of competition types (Nicholson 1954), because the two characters not only exhibited great change in the *C. maculatus*-*H. prosopidis* system but also correlate well with each other (this study; Credland et al. 1986; Toquenaga and Fujii 1990).

On day 270, or the 13th generation of the host, three females of the host species were each sampled from a randomly chosen replicate of **HP**₂ and **HO** (replicate 1) systems, and allowed to oviposit on mung beans of 50–60 mg for up to 24 h. Four days after oviposition, egg densities per bean were adjusted to one, two, and four, and 11–16 beans for each egg density and were placed in wells of plastic boxes. Fresh weights of 11 emerging adults grown singly were examined for both populations. The number of emerging adults was recorded five weeks after oviposition. On day 650, adult hosts were sampled from all the persisting replicates for the same examination of the weight of their offspring when grown alone.

For the offspring of host adults sampled on day 270, fresh weight at emergence was greater in **HO** than in **HP**₂ ($F = 7.53$, $df = 1$, $P < 0.05$, Fig. 5a). There was also a difference in weight between the sexes ($F = 100$, $df = 1$, $P < 0.001$) but no interaction between populations and sex ($F = 1.96$, $df = 1$, $P > 0.1$). For the offspring of hosts sampled on day 650, all the replicates were pooled because there was no difference among them ($F = 0.614$, $df = 2$, $P > 0.5$) and the body weight was not different between **HO**

and **HP**₂ ($F = 1.33$, $df = 1$, $P > 0.5$, Fig. 5b). There was a significant difference again between sexes ($F = 376$, $df = 1$, $P < 0.001$). Interaction effect became significant between populations and sex ($F = 43$, $df = 1$, $P < 0.001$).

Density of emerging adults from beans was not different between the two populations **HO** and **HP**₂ sampled on day 270 ($F = 2.08$, $df = 1$, $P > 0.1$). The maximum density of emerging adults was 1.18 ± 0.12 (mean \pm SE). The density of hatched larvae ($F = 1.44$, $df = 2$, $P > 0.1$) and its interaction with populations ($F = 0.437$, $df = 2$, $P > 0.5$) also did not affect the density of emerging adults.

In summary, the experimental results suggested that host evolution towards the contest type, as judged from the greater adult weight, was more advanced in the absence of the parasitoid on day 270, but later on day 650 the effect of parasitoid had disappeared. The change in the maximum emergence per bean from 2.42 to 1.18 paralleled the gradual decrease in the host population sizes in replicates 1 and 3 during 400 days, or 20 host generations.

Discussion

Evolution of contest competition and evolutionary trade-offs in the host

This study is the first demonstration of character changes from one competition type to the other in a population which originated from a single founder population (Fig. 1), since contest and scramble types of the cowpea weevil *C. maculatus* have been maintained as separate established laboratory populations, or strains (Dick and Credland 1984; Thanthianga and Mitchell 1987; Toquenaga and Fujii 1990). Correlation between the degree of contest competition and increased body size found in this study

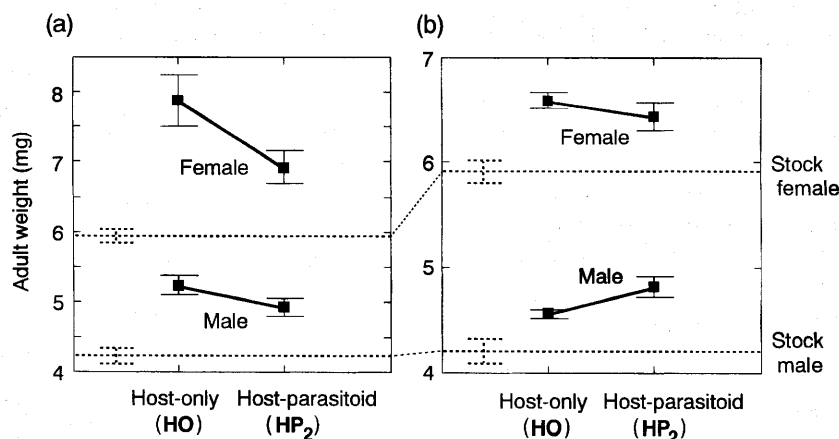


Fig. 5. Fresh weights (mean \pm SE) of emerging host adults that were sampled on (a) day 270 and (b) day 650, from a host-only (**HO**) and host-parasitoid (**HP**₂) systems. Sample sizes were five or six for each combination of sex \cdot population for day 270 and 20 for day 650. Fresh weights (mean \pm SE) for stock population (S) shown in Fig. 2 are indicated by broken lines.

(Figs. 1 and 2) have already been reported elsewhere (Credland et al. 1986; Toquenaga and Fujii 1990). Results of the present study show an evolutionary trade-off between body size and the rate of larval survival and development, suggesting a cost of contest competition on larval survivorship and development (Table 1).

Other indices for the degree of contest competition could be employed, such as the *c*-value which was developed to measure the centripetal tendency in larval burrowing behavior (Toquenaga et al. 1994). This is unless they contradict the definition of a single emerging adult per bean at any nonzero larval densities, which includes the original definition of the contest type (Nicholson 1954). Care must be taken when employing such indices for the degree of contest competition because the very trait under selection may be different among populations with different histories of natural and artificial conditions; for example, the large body size of the Yemen strain as a result of physiological adaptation (Smith 1990).

Effect of parasitoid attacks on the evolution of the host

This study also tested experimentally the two predictions of the model by Tuda and Iwasa (1998), (1) gradual decrease in population size of the host in a host-only system and (2) slower increase of the contest-type hosts in a host-parasitoid system than in a host-only system for the initial 20 generations, after which the host-parasitoid system should catch up to the host-only population in the degree of contest competition. The model assumes that the parasitoid serves as a non-selective perturbation factor, and predicts such perturbation moderates the competition among host larvae. The first prediction was supported for two out of three replicates of the host-only system, and founder effects might have been present to cause the among-replicate variation (Fig. 4).

The second prediction was supported for a higher degree of contest competition in the host-only population in the initial 20 generations and disappearance of the difference in the later generations. For the initial 20 generations, the higher degree of contest competition in the randomly-selected replicate of the host-only system correlated with a long-term decrease in population sizes, although variation among replicates was left untested. For the later generations, however, the among-replicate variation was proven negligible, and as the model predicted the degree of contest competition of the host in the host-parasitoid system converged to that of the host-only system. Therefore, parasitoid attack, or random perturbation, can slow host evolution towards the contest type, but the effect is not sufficient to reverse the intense natural selection on the host, induced by the small resource units. Furthermore, an interaction effect between sex and the presence of parasitoid appeared

and this suggests either differential parasitoid attack or sexual dimorphism in contest competition in the later generations. The reason for its appearance at this stage of the long-term experiment is open for further studies.

Bottle-neck effects on host character change were anticipated in the host-parasitoid replicate **HP₁** (Tuda and Iwasa 1998) but, judging from the population sizes of the host, was relatively negligible in the present study, especially for the sampled cohorts subjected to the experiments on the degree of contest competition (i.e. the cohorts on day 270 that trace back to generations on days 250, 230 and so on; Fig. 4).

The attack rate of the parasitoid did not change (Fig. 3), probably because of a lack of genetic diversity, which had been lost through long laboratory rearing. Further careful examination is needed to uncover other possible changes in the life history characters of the parasitoid.

Stability by the evolution of contest competition

The impact of host evolution on host-parasitoid system persistence can be predicted by a detailed simulation study on *Callosobruchus-H. prosopidis* experimental systems (Tuda 1996): Reduction in emerging adult density per bean through larval competition is predicted to increase persistence of such systems. The increase in the vulnerable period should, on the other hand, decrease persistence time. Earlier occurrence of contest competition than scramble competition is stabilizing (Tuda 1996) and a longer reproductive period with no change in total fecundity should stabilize the system (Tuda and Shimada 1995). In conclusion, it is possible that evolution towards contest-type larval behavior stabilizes the host-parasitoid system, and in the present study this is supported by a greater absolute value of negative Lyapunov exponents for the later census period. The stability, however, may not be global but local because perturbation added to the host-parasitoid system after day 810 terminated the system (M. Tuda and B. Dennis, unpublished manuscript).

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References

- Brakefield, P. M. (1987) Industrial melanism: do we have the answers? *Trends in Ecology and Evolution* 2: 117-122.
- Credland, P. F., K. M. Dick and A. W. Wright (1986) Relationships

- between larval density, adult size and egg production in cowpea seed beetle, *Callosobruchus maculatus*. *Ecological Entomology* **11**: 41–50.
- Dick, K. M. and P. F. Credland (1984) Egg production and development of three strains of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *Journal of stored Product Research* **20**: 221–227.
- Ellner, S., D. W. Nychka and A. R. Gallant (1992) LENNS, a program to estimate the dominant Lyapunov exponent of noisy non-linear systems from time series data. *Institute of Statistics Mimeo Series #2235* (BMA Series #39), Statistics Department, North Carolina State University, Raleigh NC 27695–8203.
- Nicholson, A. J. (1954) An outline of the dynamics of animal populations. *Australian Journal of Zoology* **2**: 9–65.
- Nicholson, A. J. (1957) The self-adjustment of populations to change. *Cold Spring Harbor Symposium in Quantitative Biology* **22**: 153–73.
- Reznick, D. N., H. Bryga and J. A. Endler (1990) Experimentally induced life-history evolution in a natural population. *Nature* **346**: 357–359.
- Roff, D. A. (1992) *The evolution of life histories; theory and analysis*. Chapman & Hall, New York.
- Smith, R. H. (1990) Adaptations of *Callosobruchus* species to competition. pp. 351–360. In K. Fujii, A. M. R. Gatehouse, C. D. Johnson, R. Mitchel and T. Yoshida (eds.) *Bruchids and legumes: economics, ecology and coevolution*. Kluwer, the Netherlands.
- Stearns, S. C. (1983) The genetic basis of differences in life-history traits among six stocks of mosquitofish that shared ancestors in 1905. *Evolution* **37**: 618–627.
- Stearns, S. C. (1992) *The evolution of life histories*. Oxford University Press, Oxford.
- Stokes, T. K., W. S. C. Gurney, R. M. Nisbet and S. P. Blythe (1988) Parameter evolution in a laboratory insect population. *Theoretical Population Biology* **34**: 248–265.
- Thanthianga, C. and R. Mitchell (1987) Vibration mediate prudent resource exploitation by competing larvae of the bruchid bean weevil *Callosobruchus maculatus*. *Entomologia Experimentalis et Applicata* **44**: 15–21.
- Toquenaga, Y. and K. Fujii (1990) Contest and scramble competition in *Callosobruchus maculatus* (Coleoptera: Bruchidae). I. Larval competition curves and resource sharing patterns. *Researches on Population Ecology* **32**: 199–211.
- Toquenaga, Y., M. Ichinose, T. Hoshino and K. Fujii (1994) Contest and scramble competitions in an artificial world: Genetic analysis with GA. pp. 177–99. In C. G. Langton (ed.) *Artificial Life III*. Addison-Wesley.
- Tuda, M. (1996) Temporal/spatial structure and the dynamical property of laboratory host-parasitoid systems. *Researches on Population Ecology* **38**: 133–140.
- Tuda, M. and Y. Iwasa (1998) Evolution of contest competition and its effect on host-parasitoid dynamics. *Evolutionary Ecology* **12**: (in press).
- Tuda, M. and M. Shimada (1995) Developmental schedules and persistence of experimental host-parasitoid systems at two different temperatures. *Oecologia* **103**: 283–291.
- Zar, J. H. (1984) *Biostatistical analysis*, 2nd edn. Prentice-Hall, New Jersey.

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