REFERENCES AND NOTES

1. J. L. Bos, *Cancer Res.* **49**, 4682 (1989).
2. R. Sager, *Science* **246**, 1406 (1989).
3. M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
4. A. Balmain and K. Brown, *Adv. Cancer Res.* **51**, 147 (1988).
5. A. Balmain, M. Ramsden, G. T. Bowden, J. Smith, *Nature* **307**, 658 (1984).
6. H. Zarbl, S. Sukumar, A. V. Arthur, D. Martin-Zanca, M. Barbacid, *ibid.* **315**, 382 (1985).
7. C. W. Welsch, *Cancer Res.* **45**, 3415 (1985).
8. J. Leon, H. Kamino, J. J. Steinberg, A. Pellicer, *Mol. Cell. Biol.* **8**, 786 (1988).
9. S. Sukumar, V. Notario, D. Martin-Zanca, M. Barbacid, *Nature* **306**, 658 (1983).
10. S. Sukumar, W. P. Carney, M. Barbacid, *Science* **240**, 524 (1988).
11. R. K. Saiki *et al.*, *ibid.* **239**, 487 (1988).
12. R. Kumar and M. Barbacid, *Oncogene* **3**, 647 (1988).
13. R. Kumar and L. L. Dunn, *Oncogene Res.* **4**, 235 (1989).
14. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
15. L. J. Leon, I. Guerrero, A. Pellicer, *Mol. Cell. Biol.* 7, 1535 (1987).
16. Crude cell lysates prepared from liver and spleen were tested as described in (*12*) and (*13*). No G to A mutated H-*ras* or K-*ras* oncogenes could be detected in these samples (R.K. and M.B., unpublished observations).
17. The studies were initiated in the Developmental Oncology Section, BRI-Basic Research Program, NCI-FCRF, Frederick, MD 21701. Supported in part by contract NO1-CO-74101 of the NCI, DHHS to Bionetics Research, Inc., and by grants (to S.S.) from the NCI (1-RO1-CA48943), American Cancer Society (CD-402), and Clayton Foundation. We thank P. Djuricovic for expert help in surgically removing the mammary glands, L. Dunn and T. Rowe for technical assistance, A. Sliski for critical reading of the manuscript, and J. Parton for preparation of the manuscript.

11 January 1990; accepted 20 April 1990

Enhancement of Symbioses Between Butterfly Caterpillars and Ants by Vibrational Communication

P. J. DeVries

Butterfly caterpillars produce calls that appear to play a role in maintaining symbiotic associations with ants. A survey of butterfly species from South and Central America, North America, Europe, Thailand, and Australia suggests that the ability for caterpillars to call has evolved independently at least three times, and that calling may be ubiquitous among ant-associated species. Because ants use substrate-borne sound in their communication systems, this study points to the possibility that the calls of one insect species have evolved to attract other, unrelated species.

Among butterfly caterpillars, the ability to form symbioses with ants is known only in the families Riodinidae and Lycaenidae (*1, 2*). The nature of these symbioses is that caterpillars provide ants with food secretions in exchange for protection against predators. By use of analogous organs, caterpillars from these two groups may mediate symbioses with ants by producing amino acid and sugar secretions or semiochemicals (*3, 4*). Studies indicate that if caterpillars are found by insect predators without ants, they have no chance of survival (*1, 2*). Thus, there is a premium for any caterpillar species involved in symbioses with ants to maintain a constant cadre of ant guards. In addition to secretory organs, riodinid caterpillars that form symbioses with ants often have a pair of nonsecretory structures termed vibratory papillae (*5*) whose movement has been speculated to convey vibrations to ants (*4, 6*). However, the function of vibratory papillae has never been demonstrated. I report that (i) the vibratory papillae of riodinid caterpillars function as organs for producing acoustic calls, (ii) caterpillars unable to produce calls are experimentally shown to attract fewer ants, and (iii) comparative data suggest that caterpillar calls have evolved at least three times, always as part of symbiotic associations with ants.

Caterpillar calls of a typical ant-associated riodinid butterfly, *Thisbe irenea* (*7*), were studied on Barro Colorado Island, Panama, and surrounding mainland habitats. Caterpillar calls were detected and recorded by using a particle velocity microphone and amplifier connected to a tape recorder, and the recordings were subsequently analyzed for wave form and frequency characteristics (*8*).

Third through fifth instar caterpillars of *Thisbe irenea* all produced low amplitude calls (*9*). The calls were detectable within a 5-cm radius of the caterpillar when the microphone was in contact with the recording substrate (*8*), or if held in direct contact with the caterpillar's body. When the microphone was held 1 mm away from caterpillars or substrate, no calls were detected, indicating that they were entirely substrate-borne. Caterpillars called constantly when walking or when they were prodded by the observer, and rarely when at rest; previous observations have indicated that the vibratory papillae are most frequently used at these times (*4*).

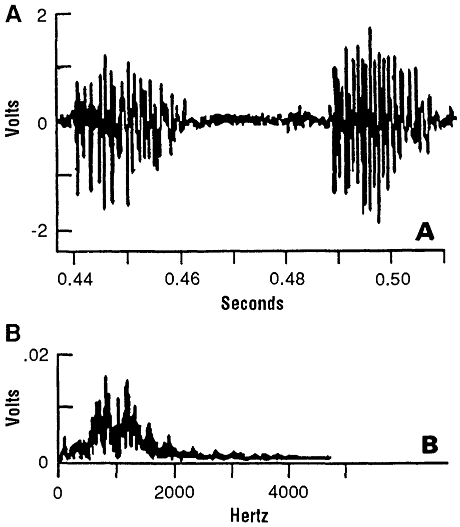

Fig. 1. Waveform of the substrate-borne call produced by a fifth instar *Thisbe irenea* caterpillar. (**A**) An oscillogram trace of two pulses of a typical call. A typical call consists of approximately 16 single pulses per second with an interval between each pulse. (**B**) An amplitude-frequency spectrum showing the dominant frequencies of a typical call.

The calls typically consisted of a simple, repeated pulse stridulation (Fig. 1). The mean pulse rate for the calls of 38 individual caterpillars (2-s segment per call) was 16.5 pulses per second (SE, 0.45; maximum, 21.7; minimum, 11.1). Slight variation in number of pulses per second occurred within and between individual caterpillars; this was probably due to the variation in walking speed of each caterpillar or the stress level while being recorded. Analyses of 76 pulses from 20 individual caterpillars showed a mean dominant frequency of 896 Hz and mean high and low frequencies of 1480 and 370 Hz, respectively (*10*).

The ability to call was eliminated in caterpillars that had their vibratory papillae removed (*11*). Except for their loss of call, these caterpillars fed, oscillated their heads, interacted with ants, pupated, and eventually produced adult butterflies. Because new vibratory papillae are produced at each instar, the ability to call returned to all mute caterpillars when they molted to the next instar. Thus, each individual caterpillar became its own control during the course of the study. Those caterpillars with only a single vibratory papilla removed all produced calls (*11*). Vibratory papillae do not develop until the third instar (*4*), and correspondingly, first and second instar caterpillars were all found to be mute. The obvious head movements associated with sound pro-

---

Department of Zoology, University of Texas, Austin, TX 78712, and Smithsonian Tropical Research Institute, APO Miami, FL 34002-0011.

duction (*9*) were not noticed in first and second instar caterpillars, but the possibility that this behavior occurs cannot be eliminated.

Caterpillars capable of calling were tended by significantly more individual ants than those caterpillars rendered mute (*12*). These results were found in both the field (median: six singing and three mute; $T(33) = 109.5$, $Z = 3.055$, $P < 0.005$), and laboratory experiments (median: 30 singing and 26 mute; $T(16) = 10.0$, $Z = 2.999$, $P < 0.005$). Because the secretory organs remained unaltered by experimental manipulation (*11*), all caterpillars, both calling and mute, were tended by ants after placement on the plants. Ants simply tended the caterpillars when they found them. However, given the survival benefits caterpillars accrue from the constant attention of ants (*1, 2*), these experiments suggest that caterpillar calls enhance ant association (*13*).

The ability to call was found in 13 other species from seven genera of ant-associated riodinid caterpillars (Table 1). All of these caterpillars possessed vibratory papillae, and all 14 species produced substrate-borne calls similar to that of *T. irenea* (*14*). An additional four species in the riodinid genus, *Eurybia*, were found to produce substrate-borne calls (Table 1) even though they did not possess vibratory papillae (*5*). Thus, mechanisms for call production have evolved at least twice within riodinid butterflies: once with the vibratory papillae, and independently through some as yet unknown means.

In contrast to ant-associated species, a survey of 19 species in 16 genera of neotropical riodinid caterpillars that neither form symbioses with ants, nor possess secretory organs, showed that none produced a detectable call (Table 1). A further survey showed that 25 species of Panamanian caterpillars from the butterfly families Papilionidae ($n = 2$), Pieridae ($n = 2$), and Nymphalidae ($n = 21$), none of which are known to associate with ants, produced no detectable calls.

Butterfly caterpillars in the family Lycaenidae do not have vibratory papillae (*5*), yet 19 lycaenid species that typically associate with ants were found to produce low amplitude, substrate-borne calls (Table 1). How these lycaenid caterpillars produce calls remains unknown. In parallel to the riodinids, the caterpillars of four neotropical lycaenid species that do not associate with ants did not call (Table 1). These surveys therefore suggest that the mechanism for calling in butterfly caterpillars has evolved three times in the context of ant association—twice in the Riodinidae, and at least once in the Lycaenidae. The discovery of calling by ant-associated caterpillars from five biogeographic regions (Table 1) is consistent with the idea that, in addition to secretory organs (*1–4*), caterpillar calls are also likely to be an important adaptation to forming symbioses with ants, especially since caterpillars of closely related species that do not form associations with ants did not call.

In using sound to mediate symbioses with ants, caterpillars may be exploiting a communication system normally used among ants. Many ants produce and respond to stridulations and substrate-borne vibrations as part of colony communication and recruitment (*15*), including the ants used in the present study. For example, the substrate-borne stridulations of a buried *Atta* ant attract nestmates who help dig it out, and stridulations of the ants *Novomessor* and *Messor* serve to recruit nestmates to food sources (*15*). Since substrate-borne vibrations are elements of ant communication systems, it is likely that the vibrational calls of butterfly caterpillars elicit an investigative response in attending ants. Thus in cases where attending ants are critical to butterfly caterpillars for surviving the attacks of predators (*1, 2*), caterpillars may use calls, in concert with their secretory organs, to recruit and maintain the presence of protective ants; calling caterpillars may accrue better protection from enemies than mute ones.

Although sound production that functions during courtship or as a defense has been reported from a variety of moth families (*16*), sound production is rarely reported from butterflies (*17*). Adult butterfly sounds are thought to function in courtship or as defenses in members of the family Nymphalidae (*18*), whereas sounds of pupae in the Lycaenidae are considered predator deterrents (*19*). However, prior to this report, sound production was unknown from butterfly caterpillars, nor was it known from any symbiotic association with ants.

Our current understanding of insect communication suggests that acoustical signals evolved in response to courtship and rivalry, mate recognition (*16, 20*), short distance communication between colony members of social insects (*15*), or as defenses (*19, 21*). In most of the cases presented here (Table 1), caterpillar calls occur in the context of mutualistic interactions with ants, yet calls may

Table 1. Call production by riodinid and lycaenid butterfly caterpillars. Myrmecophilous refers to species that typically form symbioses with ants, and nonmyrmecophilous are species that do not associate with ants. The country of origin for each species tested is in parentheses following its name. Abbreviations for countries: Pan for Panama and CR for Costa Rica. The ability of the caterpillar to produce calls is shown by Y for calls produced and N for no calls produced. Depending on the species, from 1 to more than 50 individual caterpillars were surveyed for ability to call.

Riodinidae		Lycaenidae	
Myrmecophilous	Nonmyrmecophilous	Myrmecophilous	Nonmyrmecophilous
Eurybia lycisca (Pan) Y	Leucochimona lagora (CR) N	Chlorostrymon simaethis (Pan) Y	Eumaeus godarti (Pan, CR) N
Eurybia patrona (Pan) Y	Leucochimona sp. (Pan) N	Strymon yojoa (Pan) Y	Symbiopsis tanais (Pan) N
Eurybia elvina (Pan) Y	Leucochimona iphias (Pan) N	Arawacus lincoides (Pan) Y	Thecla hisbon (Pan) N
Eurybia sp. (Ecuador) Y	Mesosemia telegone (Pan) N	Olynthus narbal (Pan) Y	Pseudolycaena damo (Pan) N
Thisbe irenea (Pan) Y	Mesosemia sp. (Ecuador) N	Therias pedusa (Pan) Y	
Synargis mycone (Pan) Y	Euselasia sp. (Pan) N	Therias nr enenia (Pan) Y	
Synargis gela (Ecuador) Y	Cremna thasus (Pan) N	Panthiades bitias (Pan) Y	
Juditha molpe (Pan) Y	Napaea eucharilla (Pan) N	Rekoa palegon (Pan) Y	
Menander menander (Pan) Y	Ancyluris inca (Pan) N	Tmolus legytha (Pan) Y	
Calospila cilissa (CR) Y	Rhetus arcius (Pan) N	Thecla sp. (CR) Y	
Calospila emylius (Ecuador) Y	Charis sp. (Pan) N	Lycaeides melissa samuelis (U.S.) Y	
Unknown genus (Pan) Y	Mesene sp. (Pan) N	Hypolycaena erylius (Thailand) Y	
Theope thestias (Pan) Y	Melanis pixie (CR) N	Jalmenus evagoras (Australia) Y	
Theope matuta (Pan) Y	Mesenopsis bryaxis (Pan) N	Maculinea arion (Poland) Y	
Theope virgilia (Pan) Y	Esthemopsis sericina (Pan) N	Maculinea rebeli (France) Y	
Theope sp. (Pan) Y	Symachia tricolor (Pan) N	Maculinea alcon (France) Y	
Theope nr. decorata (CR) Y	Sarota gyas (Pan) N	Cupido minimus (England) Y	
Nymphidium mantus (Pan) Y	Anteros formosus (Pan) N	Lysandra bellargus (England) Y	
	Emesis lucinda (CR) N	Polyommatus icarus (England) Y	

even be maintained in the face of parasitic interactions. For example, the caterpillars of at least three species of *Maculinea* (Table 1) live inside *Myrmica* ant nests to feed on the larval brood of their host ant species (*22*). Hence, this study points to the possibility that under selection for symbiotic associations, the calls of one insect species have evolved to attract other, distantly related insect species.

REFERENCES AND NOTES

1. N. E. Pierce and P. S. Mead, *Science* **211**, 1185 (1981); N. E. Pierce, R. L. Kitching, R. C. Buckley, M. F. J. Taylor, K. F. Benbow, *Behav. Ecol. Sociobiol.* **21**, 237 (1987).
2. P. J. DeVries, thesis, University of Texas, Austin, (1987); *Biol. J. Linn. Soc.*, in press.
3. N. E. Pierce, *Am. Nat.* **125**, 888 (1983); *Oxford Surv. Evol. Biol.* **4**, 89 (1987); K. Fiedler and U. Maschwitz, *Oecologia* **75**, 204 (1988); *Ethology* **80**, 71 (1989); P. J. DeVries and I. Baker, *J. N.Y. Entmol. Soc.* **97**, 332 (1989).
4. P. J. DeVries, *Zool. J. Linn. Soc.* **94**, 379 (1988).
5. C. B. Cottrell, *ibid.* **80**, 1 (1984); D. J. Harvey, thesis, University of Texas, Austin (1987).
6. G. N. Ross, *Ann. Ent. Soc. Am.* **59**, 985 (1966).
7. The third to fifth (and final) instar caterpillars of *Thisbe irenea* bear three distinct sets of specialized organs for associating with ants: (i) paired, eversible organs which secrete food to ants on segment A-8; (ii) paired, eversible organs that presumably secrete a semiochemical to ants on segment T-3; and (iii) a pair of chitinized vibratory papillae on the anterior edge of segment T-1 that, in concert with oscillating the head in and out, beat most frequently when caterpillars are walking or are stressed. First and second instar caterpillars possess none of these organs. For a detailed account of *T. irenea* caterpillar biology see DeVries (*4*).
8. Calls were detected by placing caterpillars on a taut paper membrane sandwiched between 4-inch diameter plastic rings that had a particle velocity microphone touching the paper membrane. Calls were recorded on a Marantz PMD 420 cassette tape recorder, and subsequently analyzed with a Kay DSP Model 5500 Sonograph and a Data 6000 wave form analyzer. The microphone and amplifier was built to the specifications of H. C. Bennet-Clark [*J. Exp. Biol.* **108**, 459 (1984)].
9. Caterpillar calls were all of sufficiently low amplitude as to be detectable only by employment of the particle velocity microphone. As suggested by DeVries (*4*), it is likely that calls are produced when the shafts of the vibratory papillae drag across the granulations on the head when the caterpillar oscillates its head in and out.
10. Frequencies were measured for 76 individual pulses taken from 20 individual walking caterpillars: 15 fifth instar caterpillars (11 individuals, 4 pulses; 1 individual, 3 pulses; 2 individuals, 2 pulses; and 1 individual, 1 pulse), and five fourth instar caterpillars (4 pulses each).
11. Twenty-five individual caterpillars ranging from third to fifth instar had both vibratory papillae removed with finely pointed forceps. After subsequent testing with the particle velocity microphone, all caterpillars were allowed to molt, retested for sound production, and depending on the instar, had their vibratory papillae removed again. An additional five caterpillars, all fourth instars, had only a single vibratory papilla removed and were tested for their ability to produce sound.
12. To test if *T. irenea* caterpillar calls were attractive to ants, two sets of experiments were performed with field-collected caterpillars that were paired by instar and size before experimentation. One set was performed in an ambient temperature laboratory using captive ant colonies and potted plants ($n = 16$ pairs), and the other set was performed in the field with naturally occurring plants and ant colonies ($n = 35$ pairs). In each pair of caterpillars, one individual had the vibratory papillae removed, whereas the other individual retained them. Paired caterpillars were placed on individual plants where all caterpillars and ants had been removed immediately before experimentation. The pairs were then censused simultaneously for the number of ants tending each caterpillar at time intervals ranging from 1 to 12 hours, and each pair was left on the plant for 1 to 4 days of censuses. During the study each plant had only the paired caterpillars on it. All experiments used the same species of ant, *Ectatomma ruidum* (Ponerinae), and all caterpillar pairs were either fourth or fifth instars. The cumulative numbers of ants tending caterpillar pairs were compared by a Wilcoxon matched-pairs test.
13. Quantifying the attenuation of substrate-borne signals is problematical because signal attenuation varies with substrate, the dispersion rate, frequencies, and type of waves produced and the distance from signal [See M. Gogala (*20*)]. The natural substrate caterpillars and ants live on is a combination of leaves, petioles, stems, bark, and soil—all with varied physical properties. Yet on these types of substrates, insects may receive directional information from vibrational signals with frequencies similar to those found in caterpillar calls [see M. Gogala (*20*)]. Although there is little doubt that natural substrates have different transmission properties than the substrate on which calls were recorded (*8*), it is likely (but not yet tested) that ants could receive caterpillar calls from distances of 5 cm on a variety of substrates.
14. P. J. DeVries, unpublished observations.
15. H. Markl and B. Holldöbler, *Behav. Ecol. Sociobiol.* **4**, 183 (1978); H. Markl, in *Neuroethology and Behavioral Physiology*, F. Huber and H. Markl, Eds. (Springer Verlag, Berlin, 1983), pp. 332–353; C. Baroni-Urbani, M. V. Buser, E. Schilliger, *Insect. Soc.* **35**, 241 (1988).
16. H. G. Spangler, M. D. Greenfield, A. Takessian, *Physiol. Entomol.* **9**, 87 (1984); A. Surlykke and M. Gogala, *J. Comp. Physiol.* **A159**, 267 (1986); M. Hunter, *Ecol. Entomol.* **12**, 355 (1987); J. Alcock, D. T. Gwynne, I. R. Dadour, *J. Insect. Behav.* **2**, 27 (1989).
17. R. E. Silberglied, in *How Animals Communicate*, T. A. Seebok, Ed. (Indiana Univ. Press, Bloomington, 1977), pp. 362–402.
18. G. Ross, *J. Res. Lep.* **2**, 241 (1963); B. Mohl and L. A. Miller, *J. Exp. Biol.* **64**, 639 (1976); S. Kane, *J. Lep. Soc.* **36**, 200 (1982).
19. J. C. Downey and A. C. Allyn, *Bull. Allyn Mus.* **14**, 1 (1973); J. C. Downey and A. C. Allyn, *ibid.* **48**, 1 (1978); N. W. Ellferich, *Mitt. Entom. Gesellschaft Basel* **38**, 156 (1988).
20. A. W. Ewing, in *Insect Communication*, T. Lewis, Ed. (Academic Press, London, 1984), pp. 223–240; M. J. West-Eberhardt, in *Insect Communication*, T. Lewis, Ed. (Academic Press, London, 1984), pp. 283–324; M. F. Claridge, *Annu. Rev. Entomol.* **30**, 297 (1985); M. Gogala, in *Acoustic and Vibrational Communication in Insects*, K. Kalmring and N. Elsner, Eds. (Verlag Paul Parey, Berlin, 1985), pp. 117–126; R. R. Hoy, A. Hoikkala, H. Kaneshiro, *Science* **240**, 217 (1988).
21. W. M. Masters, *Behav. Ecol. Sociobiol.* **5**, 187 (1979).
22. J. A. Thomas, G. W. Elmes, J. C. Wardlaw, M. Woyciechowski, *Oecologia* **79**, 452 (1989).

23. I thank R. Cocroft, Y. Gamarra, N. Greig, R. Robbins, and S. Rand for assistance; H. Bennet-Clark, J. Bull, K. Fiedler, B. Hawkins, I. Kitching, D. Nash, D. Promislow, M. Ryan, and F. Vollrath for reading the manuscript; G. Ballmer, K. Fiedler, N. Pierce, K. Schurian, and J. Thomas for caterpillars; and P. Harvey and R. May for logistics during my visit to Oxford. Supported by a Smithsonian postdoctoral fellowship, the American Museum of Natural History, and dedicated to W. Gray and L. Morgan.

18 December 1989; accepted 19 March 1990

Allometric Engineering: An Experimental Test of the Causes of Interpopulational Differences in Performance

BARRY SINERVO AND RAYMOND B. HUEY

Hatchling lizards (*Sceloporus occidentalis*) from a southern population are large and have high locomotor performance (speed and stamina) relative to hatchlings from northern populations. In order to determine whether differences in performance are an allometric consequence of interpopulation differences in size, yolk was removed from southern eggs, thereby producing miniaturized hatchlings equivalent in size to northern hatchlings. Miniaturized southern hatchlings no longer had higher speed than northern hatchlings, but maintained higher stamina. Interpopulation differences in speed but not in stamina are thus an allometric consequence of differences in egg size. Size manipulation adds an experimental dimension to allometric analyses.

EVER SINCE HUXLEY (*1*) FIRST DREW attention to the biological significance of relative size and shape, evolutionary and functional biologists have studied the allometric scaling of diverse morphological, physiological, and ecological traits (*2*). Allometric equations not only quantify the size dependence of a trait, but can also permit comparisons among individuals, populations, or species (*3, 4*) that differ in body size. Consequently, allometric analyses are often a key step in tests of hypotheses of trait evolution (*2–5*). Nevertheless, such analyses involve statistical, not experimental, adjustments of body size (*6*). Moreover, inferences about the proximate or mechanistic causes of dramatic differences in the intercept and slope of the allometry among taxa are risky, because many factors influence morphological and physiological traits (*7*).

Here we apply a novel method for experimentally manipulating body size, and we use this method to explore the mechanistic bases for interpopulational differences in the al-

---

B. Sinervo, Department of Integrative Biology, University of California, Berkeley, CA 94720.
R. B. Huey, Department of Zoology, NJ-15, University of Washington, Seattle, WA 98915.