Adaptation in a Plant-Hummingbird Association
Author(s): Ethan J. Temeles and W. John Kress
Reviewed work(s):
Source: *Science*, New Series, Vol. 300, No. 5619 (Apr. 25, 2003), pp. 630-633
Published by: American Association for the Advancement of Science
Stable URL: http://www.jstor.org/stable/3834361
Accessed: 19/02/2013 08:55

3. J. I. Lunine, D. J. Stevenson, Y. L. Yung, *Science* **222**, 1229 (1983).
4. C. A. Griffith, T. Owen, R. Wagener, *Icarus* **93**, 362 (1991).
5. A. Coustenis, E. Lellouch, J. P. Maillard, C. P. McKay, *Icarus* **118**, 87 (1995). Note that their weak CH_4 absorption in the 1 to 2 μm windows (least well constrained) is estimated to be 8 to 18 times greater than our values. Their absolute albedos are ~25 to 50% higher than ours.
6. Haze optical constants come from Khare *et al.* (*39*).
7. Spectra of decade-old tholins (*40*) differ, at 1.2 to 1.4 μm, from spectra of young tholins (*41*). It is not clear whether the tholin chemistry proceeded in isolation or through contamination.
8. Noll *et al.* (*42*) do not consider scattering and emission, causing slightly different results from ours at 5 μm.
9. C. A. Griffith, T. Owen, G. A Miller, T. R. Geballe, *Nature* **395**, 575 (1998). Note that their 1.3 μm surface albedo is incorrect because of faulty normalization of previous observations.
10. J. T. Rayner *et al.*, *Publ. Astron. Soc. Pac.* **115**, 362 (2003).
11. C. A. Griffith, J. L Hall, T. R. Geballe, *Science* **290**, 509 (2000).
12. O. B. Toon, C. P. McKay, C. A. Griffith, R. P. Turco, *Icarus* **95**, 24 (1992).
13. C. P. McKay, J. B. Pollack, R. Courtin, *Icarus* **80**, 23 (1989).
14. E. Karkoschka, *Icarus* **133**, 134 (1998).
15. E. Karkoschka, *Icarus* **111**, 174 (1994).
16. We adopted a discrete ordinates calculation having eight streams, as described by Stamnes *et al.* (*43*).
17. Titan's thermal profile derives from Lellouch *et al.* (*44*) and Yelle *et al.* (*45*).
18. Absorption coefficients of CH_4 and CO derive from the line parameters of Husson *et al.* (*46*).
19. We varied the vertical optical depth at two altitude regions (40 to 100 km and 100 to 160 km) to derive the range of haze optical depths that fit Titan's spectrum. Haze below ~40 km is limited by the visibility of Titan's surface at 0.63 μm.
20. Our surface albedos are thus unaffected by variations in the 0.01 shape parameter (or width) of our log-normal particle distribution, the tholin optical constants (*6*), and details of the haze models.
21. K. Rages, J. B. Pollack, P. H. Smith, *J. Geophys. Res.* **88**, 8721 (1983).
22. P. H. Smith *et al.*, *Icarus* **119**, 336 (1996).
23. J. Richardson, R. D. Lorenz, A. McEwen, in preparation.
24. M. G. Tomasko, P. H. Smith, *Icarus* **51**, 65 (1982).
25. R. A. West *et al.*, *J. Geophys. Res.* **88**, 8699 (1983).
26. R. A. West, P. H. Smith, *Icarus* **90**, 330 (1991).
27. P. Rannou, M. Cabane, R. Botet, E. Chassefière, *J. Geophys. Res.* **102**, 10997 (1997).
28. P. Rannou *et al.*, *Icarus* **118**, 355 (1997).
29. C. P. McKay *et al.*, *Planet. Space Sci.* **49**, 79 (2001).
30. M. G. Tomasko *et al.*, in *Huygens: Science Payload and Mission, Vol. ESA-SP 1177*, A. Wilson, Ed. (European Space Agency Publications Division, Noordwijk, Netherlands, 1997), pp. 109–138.
31. One model that fits our optical depths contains haze above 90-km altitude, where the radius (in micrometers), column abundance (km amagat) and 0.94-μm optical depth are specified by the values (*a*, *b*, *c*) of (6.8, –424, 0), (152.3, –131.7, –34.5), and (104.3, –108.9, –20.2), respectively. These haze parameters, *x*, depend on altitude, *z* (in km), as: $z = a + b \log_{10}(x) + c[\log_{10}(x)]^2$.
32. B. Lutz, C. de Bergh, T. Owen, *Nature* **220**, 1374 (1983).
33. T. Hidayat *et al.*, *Icarus* **133**, 109 (1998).
34. Methane is assumed to be subsaturated at the surface with a humidity of 60%. A constant mixing ratio is adopted above the surface until the level of saturation is reached. Above this level, the atmosphere is saturated up to the tropopause. The mixing ratio is constant at the tropopause value above this point. An amagat is a unit of density equal to 2.69×10^{19} cm^{-3}.
35. M. Combes *et al.*, *Icarus* **129**, 482 (1997).
36. R. Meier, B. A. Smith, T. C. Owen, R. J. Terrile, *Icarus* **145**, 462 (2000).
37. S. G. Gibbard *et al.*, *Icarus* **139**, 189 (1991).
38. Note that 5-m-deep pools of methane absorb 95% of 1.1- to 5-μm sunlight (*47*).
39. B. N. Khare, C. Sagan, E. T. Arakawa, F. Suits, T. A. Callcott, M. W. Williams *Icarus* **60**, 127 (1984).
40. T. L. Roush, J. B. Dalton, Conf. 33, abstract 1532, Lunar Planetary Inst., Houston, TX (2002).
41. D. Cruikshank *et al.*, *Icarus* **94**, 345 (1991).
42. K. S. Noll, T. R. Geballe, R. F. Knacke, Y. J. Pendleton, *Icarus* **124**, 625 (1996).
43. K. Stamnes, S. Tsay, W. Wiscombe, K. Jayweera, *Appl. Opt.* **27**, 2502 (1988).
44. E. Lellouch *et al.*, *Icarus* **79**, 328 (1989).
45. R. V. Yelle, D. F. Strobell, E. Lellouch, D. Gautier, in *Huygens: Science Payload and Mission, Vol. ESA-SP 1177*, A. Wilson, Ed. (European Space Agency Publications Division, Noordwijk, Netherlands, 1997), pp. 243–256.
46. N. Husson, B. Bonnet, N. A. Scott, A. Chédin, *The "GEISA" data bank 1991 version* (Internal note L.M.D. 163, Laboratoire de Météorologie Dynamique, Palaiseau, France, 1991).
47. W. M. Grundy, B. Schmitt, E. Quirico, *Icarus* **155**, 486 (2002).
48. M. T. Lemmon, E. Karkoschka, M. Tomasko, *Icarus* **103**, 329 (1993).
49. C. A. Griffith, *Nature* **364**, 511 (1993).
50. T. B. McCord *et al.*, *J. Geophys. Res.* **103**, 8603 (1998).
51. T. B. McCord, personal communication.

52. We thank D. Cruikshank for helpful discussions regarding the spectroscopic characteristics of complex organic molecules. This research is supported by the NASA Planetary Astronomy Program.

24 December 2002; accepted 24 March 2003

Adaptation in a Plant-Hummingbird Association

Ethan J. Temeles[1]* and W. John Kress[2]

Sexual dimorphism in bill morphology and body size of the Caribbean purple-throated carib hummingbird is associated with a reversal in floral dimorphism of its *Heliconia* food plants. This hummingbird is the sole pollinator of *H. caribaea* and *H. bihai*, with flowers of the former corresponding to the short, straight bills of males, the larger sex, and flowers of the latter corresponding to the long, curved bills of females. On St. Lucia, *H. bihai* compensates for the rarity of *H. caribaea* by evolving a second color morph with flowers that match the bills of males, whereas on Dominica, *H. caribaea* evolves a second color morph with flowers that match the bills of females. The nectar rewards of all *Heliconia* morphs are consistent with each sex's choice of the morph that corresponds to its bill morphology and energy requirements, supporting the hypothesis that feeding preferences have driven their coadaptation.

Understanding natural adaptations of organisms to their environment has been the focus of evolutionary investigations since the time of Darwin (*1*) and Wallace (*2*). Plant-animal interactions, especially those between flowering plants and their animal pollinators, provide classic examples of hypothesized coadaptations that confer advantages to both mutualists (*3*, *4*). The most convincing investigations of adaptation require (i) the presence of discrete character polymorphisms in natural populations, (ii) evidence for the genetic basis of this variation, (iii) fitness measures, and (iv) comparative studies among populations (*5*). Here we present comparative data from two Lesser Antillean islands on contemporary character polymorphisms in sympatric species of the tropical plant genus *Heliconia* and their common pollinator, the purple-throated carib hummingbird, which support the principle of coadaptation between these mutualists.

The purple-throated carib hummingbird, *Eulampis jugularis*, is an example of sexual dimorphism that results from ecological causation: Although the wings and body masses of males average 8.6 and 25%, respectively, larger than those of females, the bills of females average 30% longer than those of males and are 100% more strongly curved (*6*, *7*). Temeles *et al.* (*7*) showed that *E. jugularis* is the sole pollinator of two species of *Heliconia* on St. Lucia, a green-bracted *H. bihai* whose long, curved flowers match the bills of females and a red-bracted *H. caribaea* whose short, straight flowers match the bills of males (Fig. 1, A to E and G). Each sex prefers and feeds most efficiently from the *Heliconia* species whose flowers correspond to its bill size and shape, which is consistent with the predictions of ecological causation and feeding adaptation (*7*). At forest reserves on St. Lucia where the red-bracted *H. caribaea* is absent or rare, a red-green–bracted morph of *H. bihai* takes its place and has shorter, straighter flowers with a morphology intermediate between those that correspond to the bill morphologies of males and females (Fig. 1F). Male *E. jugularis* at these sites are associated primarily with the red-green–bracted morph of *H. bihai*,

---

[1]Department of Biology, Amherst College, Amherst, MA 01002 USA. [2]Botany, MRC-166, United States National Herbarium, National Museum of Natural History, Post Office Box 37012, Smithsonian Institution, Washington, DC 20013–7012 USA.

*To whom correspondence should be addressed. E-mail: ejtemeles@amherst.edu

Science

25 April 2003

Vol. 300 No. 5619
Pages 533–684 $10

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

whereas females visit both the red-green– and green-bracted morphs.

E. jugularis on St. Lucia provides compelling evidence for a tight coevolutionary association between sexual dimorphism in its bill morphology and the floral morphology of its *Heliconia* food plants. We tested the generalities of this plant-pollinator system with comparative field studies on a second Antillean island, Dominica.

Our visits to Dominica in April of 2001 and 2002 coincided with peak flowering of the *Heliconia* species and the breeding season of the hummingbirds. We conducted roadside censuses of *Heliconia* species along six transects (*8*). As on St. Lucia, the principal understory food plants of *E. jugularis* on Dominica are *H. bihai* and *H. caribaea*. In contrast to their sympatric distribution on St. Lucia, however, on Dominica the two species occur allopatrically along an altitudinal gradient, with *H. caribaea* occupying lower elevations (100 to 600 m) and *H. bihai* occupying higher elevations (600 to 900 m). In addition, the pattern of floral dimorphism on Dominica is completely reversed from the pattern on St. Lucia: *H. bihai*, which has two color morphs on St. Lucia (green or red-green), has only one color morph on Dominica (red with a yellow stripe) (Fig. 1H). In contrast, *H. caribaea*, which has only one color morph on St. Lucia (red), has two color morphs on Dominica (red or yellow) (Fig. 1, I and J).

To determine whether the sexes of *E. jugularis* differed in their use of these two *Heliconia* species, we watched birds at patches of both species of *Heliconia* (*8*). Males were associated exclusively with *H. caribaea*, which they defended against conspecifics, and were not observed to visit *H. bihai*, although they fed from the flowers of other plants at sites where *H. bihai* was common. In contrast, females fed at *H. caribaea* and were the sole visitors to *H. bihai* (20 of 49 females, but 0 of 21 males, were observed to visit *H. bihai*; $P < 0.001$; $\chi^2 = 12.0$; df = 1).

To examine the relationship between flower use and bill morphology, we measured flower lengths and curvatures of *H. bihai* and the two *H. caribaea* morphs along four transects having all three kinds of *Heliconia* (Table 1). For all four transects, the flowers of *H. bihai* were significantly longer and more strongly curved than the flowers of either morph of *H. caribaea*, consistent with our observations of feeding preferences [$P < 0.05$; one-way analyses of variance (ANOVAs) with Tukey multiple comparisons].

Further support for the hypothesis of floral specialization by males and females came from an analysis of the floral morphology and visitation frequencies to the two color morphs of *H. caribaea*. At four of our six study sites (Salisbury Loop, Mt. Diablotin, Freshwater Lake, and Morne Trois Pitons) (Table 1), flowers of the red morph were slightly but significantly longer and more strongly curved than flowers of the yellow morph ($P < 0.05$; one-way ANOVAs with Tukey multiple comparisons). On the basis of Temeles *et al.*'s (*7*) finding that preferences by male and female *E. jugularis* maintain a floral dimorphism in *H. bihai* on St. Lucia, we hypothesized that a similar difference in preferences between the sexes maintained the floral dimorphism of *H. caribaea* on Dominica. The four sites at which flowers of the red morph were longer and more curved than flowers of the yellow morph included contact zones between *H. caribaea* and *H. bihai*. Because female *E. jugularis* were the sole pollinator of the red-and-yellow–striped *H. bihai* on Dominica, we hypothesized that the longer, more curved flowers of the red morph of *H. caribaea* might result from greater use by females of the red morph at sites where the two *Heliconia* species came into contact. In support of this hypothesis, 15 of 19 females were associated with red clumps of *H. caribaea* at contact zones, whereas only 2 of 13 males were associated with red clumps of *H. caribaea* at contact zones ($P < 0.0005$, $\chi^2 = 12.5$; df = 1). At low elevation sites lacking a contact zone between *H. bihai* and *H. caribaea*, equal proportions of males and females visited the red and yellow morphs (n = 8 males and 8 females).

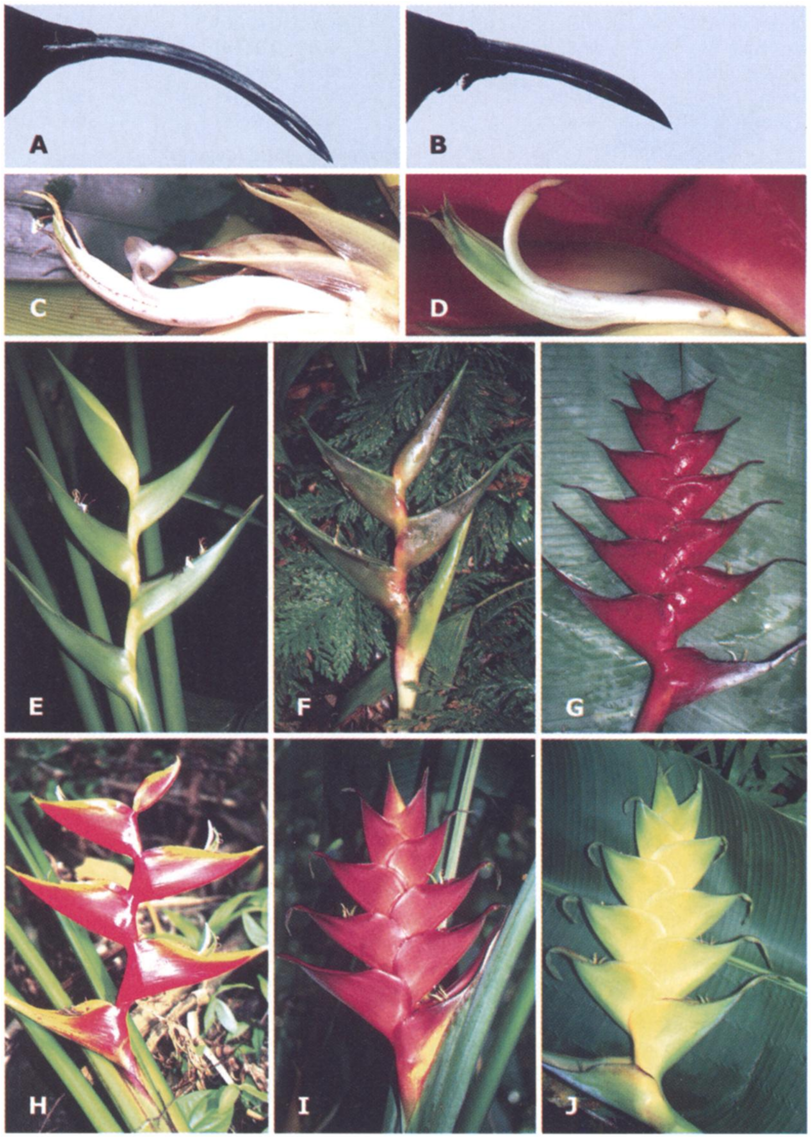

Fig. 1. Polymorphisms in bills of *E. jugularis* (A and B) and in flowers (C and D) and inflorescences (E to J) of *Heliconia* species on St. Lucia (E to G) and Dominica (C to D and H to J), West Indies. **(A)** *E. jugularis*, female bill. **(B)** *E. jugularis*, male bill. **(C)** *H. bihai*, flower. **(D)** *H. caribaea*, flower. **(E)** *H. bihai*, green inflorescence morph, St. Lucia. **(F)** *H. bihai*, red-green inflorescence morph, St. Lucia. **(G)** *H. caribaea*, inflorescence, St. Lucia. **(H)** *H. bihai*, red-and-yellow–striped inflorescence, Dominica. **(I)** *H. caribaea*, red inflorescence morph, Dominica. **(J)** *H. caribaea*, yellow inflorescence morph, Dominica.

The sexual differences in bill morphology of *E. jugularis* may result from resource partitioning of *Heliconia* species on the basis of patch rewards (*7*): Larger males, with correspondingly higher energy requirements (*8*, *9*), dominate females and establish priority of access to the more effusive *H. caribaea*. Comparisons of bract numbers—a measure of flower numbers per inflorescence (*8*)—and energy rewards among and between *Heliconia* species and morphs on Dominica and St. Lucia supported this hypothesis. On both islands, *H. caribaea* had significantly more bracts per inflorescence than *H. bihai* and at seven of eight sites offered a higher energy reward per inflorescence, consistent with male preferences for flowers of this species ($P < 0.05$; one-way ANOVAs with Tukey multiple comparisons) (Tables 2 and 3). On Dominica, the red-bracted morph of *H. caribaea* had significantly fewer bracts per inflorescence than the yellow-bracted morph, but only at sites where it came into contact with *H. bihai* ($P < 0.05$; one-way ANOVAs with Tukey multiple comparisons) (Table 2). The reduction in bract numbers of the red-bracted morph at these sites is consistent with greater use of the red-bracted morph by smaller female *E. jugularis* with their correspondingly lower energy requirements. In contrast, on St. Lucia, the red-green morph of *H. bihai* had significantly more bracts per inflorescence than the green-bracted morph, but only at sites where it geographically replaced *H. caribaea* and was used by larger male *E. jugularis*, with their correspondingly higher energy requirements ($P < 0.05$; one-way ANOVAs with Tukey multiple comparisons) (Table 3).

Table 1. Flower lengths and curvatures [mean ± SE (*n*, where *n* is the number of flowers)] of *H. bihai* and the red and yellow morphs of *H. caribaea* along six transects in Dominica, West Indies.

Transect	*H. bihai*	Red *H. caribaea*	Yellow *H. caribaea*
		Flower length (mm)	
Salisbury Loop	47.6 ± 0.3 (16)	39.7 ± 0.4 (23)	36.2 ± 0.3 (15)
Mt. Diablotin	47.2 ± 1.2 (8)	40.0 ± 0.4 (20)	37.2 ± 0.2 (15)
Freshwater Lake	48.5 ± 0.4 (25)	38.3 ± 0.3 (14)	36.6 ± 0.4 (17)
Morne Trois Pitons	48.9 ± 0.7 (13)	38.1 ± 0.2 (16)	35.4 ± 0.3 (9)
Layou River	Absent	36.4 ± 0.2 (19)	35.8 ± 0.2 (15)
Central Forest	Absent	36.2 ± 0.4 (16)	36.4 ± 0.4 (12)
		Flower curvature (degrees)	
Salisbury Loop	28.8 ± 0.5 (16)	24.0 ± 0.7 (12)	20.2 ± 0.4 (13)
Mt. Diablotin	30.0 ± 0.4 (8)	22.6 ± 0.6 (15)	19.4 ± 0.7 (12)
Freshwater Lake	29.7 ± 0.5 (12)	24.9 ± 0.6 (12)	18.4 ± 0.6 (10)
Morne Trois Pitons	31.3 ± 0.9 (13)	23.9 ± 0.6 (16)	20.7 ± 0.5 (9)
Layou River	Absent	20.0 ± 0.3 (19)	19.2 ± 0.3 (15)
Central Forest	Absent	20.8 ± 0.4 (7)	20.4 ± 0.5 (7)

Table 2. Number of bracts [mean ± SE (*n*, where *n* is the number of plants)] and joules per inflorescence of *H. bihai* and the red and yellow morphs of *H. caribaea* along six transects with and without contact zones between *H. bihai* and *H. caribaea* on Dominica, West Indies. Calculations of energy rewards in joules were based on measured average sucrose concentrations per flower of 22% and average 24-hour volumes per flower of 80, 90, and 125 μl for the yellow, red, and *H. bihai* morphs, respectively.

Transect	*H. bihai*		Red *H. caribaea*		Yellow *H. caribaea*	
	Bracts	Joules	Bracts	Joules	Bracts	Joules
			Contact zone present			
Salisbury Loop	4.3 ± 0.2 (51)	2128	6.0 ± 0.2 (52)	2138	7.3 ± 0.4 (31)	2312
Mt. Diablotin	4.8 ± 0.1 (34)	2376	6.3 ± 0.2 (76)	2245	7.4 ± 0.3 (45)	2344
Freshwater Lake	3.6 ± 0.1 (92)	1782	5.5 ± 0.2 (55)	1960	6.3 ± 0.2 (66)	1996
Morne Trois Pitons	3.5 ± 0.6 (26)	1732	7.0 ± 0.3 (30)	2494	7.9 ± 0.2 (32)	2503
			Contact zone absent			
Layou River	Absent		5.2 ± 0.3 (41)	1853	4.6 ± 0.4 (14)	1457
Central Forest	Absent		8.3 ± 0.3 (46)	2958	7.2 ± 0.5 (13)	2280

Table 3. Number of bracts [mean ± SE (*n*, where *n* is the number of plants)] and joules per inflorescence of *H. caribaea* and the red-green and green morphs of *H. bihai* at four reserves where *H. caribaea* was common or rare on St. Lucia, West Indies. Calculations of energy rewards in joules were based on measured nectar concentrations of 25, 27.5, and 29.5% sucrose and 24-hour volumes of 98.3, 88.8, and 88.9 μl for *H. caribaea*, the red-green morph, and the green morph, respectively.

Reserve	Green *H. bihai*		Red-green *H. bihai*		*H. caribaea*	
	Bracts	Joules	Bracts	Joules	Bracts	Joules
			H. caribaea *common*			
Quilesse	3.9 ± 0.1 (863)	1900	4.1 ± 0.5 (10)	1843	9.6 ± 0.2 (161)	4304
Barre de l'Isle	3.7 ± 0.1 (171)	1803	4.2 ± 0.5 (26)	1887	9.4 ± 0.2 (150)	4125
			H. caribaea *rare*			
Des Cartiers	4.3 ± 0.1 (610)	2095	5.0 ± 0.2 (186)	2247	8.0 ± 0.4 (18)	3787
Forestière	3.5 ± 0.1 (261)	1705	4.6 ± 0.2 (103)	2067	Absent	

The complete reversal between St. Lucia and Dominica in floral dimorphism of the two *Heliconia* species together with an increase in the length and curvature of flowers of the red *H. caribaea* morph to match the bills of females provides a natural experiment on the two islands for detecting coadaptation between bill morphology of *E. jugularis* and the size and shape of *Heliconia* flowers. The corresponding decrease in bract numbers and energy rewards of the red morph of *H. caribaea* on Dominica and increase in bract numbers and energy rewards of the red-green morph of *H. bihai* on St. Lucia also suggest that sexual differences in body size of *E. jugularis*, and not just bill morphology, are maintained by feeding specialization on the different *Heliconia* food plants. Because male *E. jugularis* defend the same food plants in the breeding and nonbreeding periods, both natural and sexual selection may play roles in the evolution of their larger size. Nonetheless, we believe that the ecological differences in the *Heliconia* food plants drive the feeding and mating strategies as well as the sexual differences in size, morphology, and behavior of *E. jugularis*.

Sexual dimorphism in bill length and body size is common in hummingbirds, and in many species the pattern of dimorphism in bill length is opposite the pattern of dimorphism in body size, as in *E. jugularis* (*10*, *11*). In many of these species, however, bill dimorphism is tied to sexual dichromatism in plumage, with brighter males having shorter bills and duller females having longer bills (*10*). These differences in bill morphology and plumage may be associated with sexual differences in dominance and resource use: Dominant males claim access to nectar-rich dense patches of short flowers and subordinate females are left to forage at nectar-poor, scattered resources (*10*). Longer bills may allow females to feed from a broader range of flower lengths than males (*10*). Studies of other hummingbird species (*12–14*) and *E. jugularis* support this hypothesis, but indicate that sexual differences in plumage are not always linked to sexual differences in bill morphology, resource use, or dominance.

Dimorphisms of bill and body size in *E. jugularis* are among the most extreme of any hummingbird species, and their evolution may be the result of the paucity of competing species on islands relative to mainland hummingbird communities (*15*). Such "ecological release" has been observed in many island species, including hummingbirds, although its expression does not always involve sexual dimorphisms (*16*, *17*). In hummingbirds, sexual dimorphism has been hypothesized to reflect interactions between social systems and feeding ecology (*10*, *11*); our studies of *E. jugularis* support this hypothesis. Whether other species of hummingbirds with less extreme sexual dimorphisms exhibit similar tight coadaptations with their food plants requires further study, but given the widespread occurrence of sexual differences in bill length and body size within this group of birds, the role of feeding ecology in the evolution of sexual differences may be more common than has been previously thought.

References and Notes

1. C. Darwin, *The Origin of Species by Means of Natural Selection* (John Murray, London, 1859).
2. A. R. Wallace, *Contributions to the Theory of Natural Selection* (Macmillan, London, 1871).
3. K. Faegri, L. van der Pijl, *The Principles of Pollination Ecology* (Pergamon, Oxford, 1966).
4. D. W. Schemske, C. C. Horvitz, *Science* **225**, 519 (1984).
5. D. Reznick, J. Travis, in *Adaptation*, M. R. Rose, G. V. Lauder, Eds. (Academic Press, New York, 1996), pp. 243–289.
6. L. L. Wolf, *Auk* **92**, 511 (1975).
7. E. J. Temeles, I. L. Pan, J. L. Brennan, J. N. Horwitt, *Science* **289**, 441 (2000).
8. Materials and methods are available as supporting material on *Science* Online.
9. L. L. Wolf, F. R. Hainsworth, *Ecology* **52**, 980 (1971).
10. R. Bleiweiss, *Proc. R. Soc. London Ser. B* **266**, 2491 (1999).
11. R. K. Colwell, *Am. Nat.* **156**, 495 (2000).
12. E. J. Temeles, W. M. Roberts, *Oecologia* **94**, 87 (1993).
13. F. L. Carpenter, M. A. Hixon, R. W. Russell, D. C. Paton, E. J. Temeles, *Behav. Ecol. Sociobiol.* **33**, 297 (1993).
14. F. L. Carpenter, M. A. Hixon, E. J. Temeles, R. W. Russell, D. C. Paton, *Behav. Ecol. Sociobiol.* **33**, 305 (1993).
15. P. Feinsinger, *Ecol. Monogr.* **46**, 257 (1976).
16. R. K. Selander, *Condor* **68**, 113 (1966).
17. P. Feinsinger, L. A. Swarm, *Ecology* **63**, 1574 (1982).
18. We thank D. Anthony and A. Dornelly of the St. Lucia Forestry Department and D. Williams of the Forestry and Wildlife Division of Dominica for advice and cooperation, I. Lopez and J. Clark for field assistance, and R. Colwell for comments. Supported by grants from Amherst College and NSF (grant no. IBN-0078483) (E.J.T.) and the Smithsonian Institution (W.J.K.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5619/630/DC1

Materials and Methods

31 October 2002; accepted 28 February 2003

Roles of NPM2 in Chromatin and Nucleolar Organization in Oocytes and Embryos

Kathleen H. Burns,[1,2]* Maria M. Viveiros,[4]*† Yongsheng Ren,[1]*
Pei Wang,[1,3] Francesco J. DeMayo,[3]
Donald E. Frail,[5]‡ John J. Eppig,[4] Martin M. Matzuk[1,2,3]§

Upon fertilization, remodeling of condensed maternal and paternal gamete DNA occurs to form the diploid genome. In *Xenopus laevis*, nucleoplasmin 2 (NPM2) decondenses sperm DNA in vitro. To study chromatin remodeling in vivo, we isolated mammalian NPM2 orthologs. Mouse NPM2 accumulates in oocyte nuclei and persists in preimplantation embryos. *Npm2* knockout females have fertility defects owing to failed preimplantation embryo development. Although sperm DNA decondensation proceeds without NPM2, abnormalities are evident in oocyte and early embryonic nuclei. These defects include an absence of coalesced nucleolar structures and loss of heterochromatin and deacetylated histone H3 that normally circumscribe nucleoli in oocytes and early embryos, respectively. Thus, *Npm2* is a maternal effect gene critical for nuclear and nucleolar organization and embryonic development.

A crucial step in zygotic development is the decondensation and reorganization of chromatin of male and female gametes, a process wholly dependent on factors produced during oogenesis. In vitro, *Xenopus* (x) NPM2 (NPL), an oocyte-specific nuclear protein, removes sperm protamines and facilitates nucleosome assembly and replication of the paternal genome (*1–6*). Although nucleoplasmin and related chaperones involved in chromatin remodeling are predicted to be conserved throughout evolution because oocytes can efficiently remodel heterologous sperm or somatic cell nuclei into pronuclei (*7–9*), mammalian NPM2 orthologs have until now eluded researchers.

To identify novel genes that function in oogenesis and as maternal effect genes, we engineered a polymerase chain reaction suppression-subtraction ovary library (*10*).

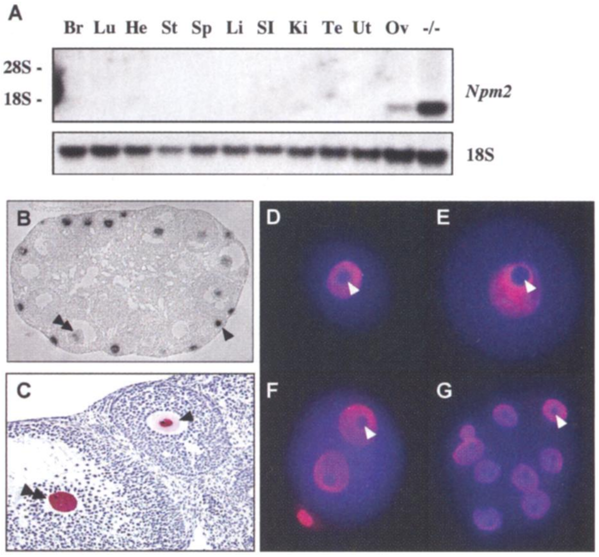

Fig. 1. *Npm2* mRNA and NPM2 protein expression. (**A**) Northern blot analysis of mouse *Npm2* and 18*S* rRNA (loading control). Br, brain; Lu, lung; He, heart; St, stomach; Sp, spleen; Li, liver; SI, small intestine; Ki, kidney; Te, testes; Ut, uterus; Ov, wild-type ovary; −/−, *Gdf9*$^{-/-}$ ovary. (**B** and **C**) In situ hybridization (B) and immunohistochemistry (C) of ovaries detect *Npm2* mRNA and protein (red staining), respectively, in all growing oocytes from the primary follicle (arrowhead) through antral follicle (double arrowhead) stages. (**D** to **G**) Immunofluorescence analysis of oocytes from 10-day-old mice (D) and adult pregnant mare serum gonadotropin (PMSG)-treated mice (E) shows that NPM2 protein is in the nucleus and excluded from the nucleolus (arrowhead). After fertilization, NPM2 localizes to pronuclei and polar bodies of one-cell zygotes (F) and persists through the eight-cell stage (G).

---

[1]Department of Pathology, [2]Molecular and Human Genetics, [3]Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. [4]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA. [5]Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA.

*These authors contributed equally to this work.

†Present address: Department of Animal Biology, Center for Transgenesis and Germ Cell Research, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348, USA.

‡Present address: CNS Discovery Research, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49007, USA.

§To whom correspondence should be addressed. E-mail: mmatzuk@bcm.tmc.edu