A Role for Ecotones in Generating Rainforest Biodiversity

Thomas B. Smith,* Robert K. Wayne, Derek J. Girman, Michael W. Bruford

Gene flow and morphological divergence were measured among 12 populations of a common species of rainforest passerine. Populations in the forest and the ecotone (the transition zone between the African rainforest and savanna) are morphologically divergent, despite high gene flow, and morphological differences between habitats are as large as those found between related species. In contrast to past theories of rainforest speciation, which emphasize geographic isolation, these results suggest that natural selection may play an important role in generating rainforest biodiversity. Because ecotone habitats may be a source of evolutionary novelty, greater attention should be paid to their conservation in order to preserve the processes that may be important to maintain rainforest diversity.

The exchange between populations of individuals who succeed in reproducing, which is commonly called gene flow, is widely viewed as a constraining force on evolution by natural selection (*1*, *2*). Consequently, most theories of differentiation and speciation have focused on isolated or partially isolated small populations in which founder effect, drift, inbreeding, and selection interact to cause evolutionary change that is free from the homogenizing effects of gene flow (*3*). Frequently, selection is implicitly assumed to be too weak to cause appreciable morphological change, given modest rates of immigration [but see (*2*, *4*)]. However, few studies have estimated levels of gene flow and divergent selection simultaneously (*2*). The vast transition zone or ecotone between the African rainforest and savanna, which is often greater than 1000 km wide (*5*), offers a unique model system to test ideas about the influence of selection on phenotypic divergence. Populations existing in forest patches in ecotones should experience divergent selection relative to central rainforest populations because these regions have smaller amounts of rainfall and experience greater annual fluctuations in environmental variables (*6*). For many rainforest bird species, food quality and habitat structure in ecotones differ dramatically from those in the central forest (*7*, *8*), which suggests that selection might explain the widespread occurrence of phenotypically unique ecotone populations and races (*8*–*10*). However, phenotypic divergence in ecotones may have a variety of causes. If ecotone forests have been genetically isolated, phylogenetic history, founder effect, genetic drift, and inbreeding could have contributed substantially to the observed pattern of divergence. In contrast, if gene flow has been high—in excess of a few migrants per generation (*1*)—then selection is strongly implicated as the cause of phenotypic divergence.

We examined morphologic and genetic divergence between six ecotone and six central rainforest populations of the little greenbul (*Andropadus virens*) (Fig. 1) (*11*). We measured divergence in five morphologic characters (Table 1) that have been shown to have high heritability in birds (*12*) and that have a close correlation with feeding ecology, flight performance, and fitness (*7*, *13*). Genetic exchange within and between regions was based on allele frequencies of eight microsatellite loci and was expressed as the parameter *Nm*, the number of migrants per generation (Fig. 2). The central forest and ecotone populations differ significantly in four of five morphological characters (Table 1), whereas only one trait differed significantly between the ecotone sites and two traits differed significantly between the forest sites. The larger values of wing length in ecotone forests are consistent with the need for greater aerodynamic efficiency in more open habitats because of increased vulnerability to aerial predators (*14*). Similarly, the larger values of bill depth are likely to reflect differences in the feeding performance and relative fitness of ecotone phenotypes (*7*). Upper mandible length does not differ significantly between forest and ecotone sites, which suggests that body size differences alone do not explain the larger values of wing length and bill depth in ecotones.

Large values of morphologic divergence may reflect selection, phylogenetic history, genetic drift, founder effect, or inbreeding in physically isolated ecotones or a combination of these factors. To address this, we compared pairwise values of multidimensional morphologic distance with pairwise values of *Nm*. Assuming an island model of migration, *Nm* can be related to F_{st}, the correlation between random gametes within a population relative to gametes of the total population, as $1/(1 + 4Nm)$ (*15*). We calculated pairwise values of G_{st}, an extension of F_{st} for multiple loci with multiple alleles (*16*). We computed morphologic divergence as the multidimensional Euclidean distance between population means of normalized measurements of five characters (Table 1). Values of *Nm* were large among all populations, generally between one and eight migrants per generation (Fig. 2). Multivariate morphological divergence between ecotone and forest populations was three to five times greater than that between ecotone-ecotone or forest-forest populations when gene flow was low ($Nm <$ two migrants per generation) but decreased to a similar value when gene flow was high ($Nm >$ seven migrants per generation). The correlation between *Nm* and morphologic divergence (Fig. 2) is significant for ecotone-forest populations ($r = -0.58$ and $P < 0.004$ for Mantel's test), with the regression line $y = -0.22x + 4.08$, but is nonsignificant for forest-forest or eco-

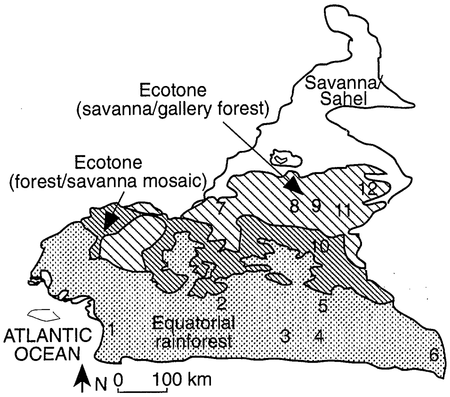

Fig. 1. Map of Cameroon showing the ecotone and forest habitats (*21*) and the locations of the 12 sampling sites (*11*). Ecotone populations are those existing in forest patches embedded in savanna habitats. Forest populations are those existing in the contiguous rainforest. The sampling sites in the contiguous forest were at least 10 km from major roads or large human settlements; the sampling sites in the ecotone consisted of patches of rainforest surrounded by savanna. These often, but not exclusively, consisted of gallery forests.

T. B. Smith, Department of Biology, San Francisco State University, San Francisco, CA 94132, USA, and Center for Population Biology, University of California, Davis, CA 95616, USA.
R. K. Wayne, Department of Biology, University of California, Los Angeles, CA 90024, USA.
D. J. Girman, Romberg-Tiburon Center, San Francisco State University, San Francisco, CA 94920 USA, and Center for Population Biology, University of California, Davis, CA 95616, USA.
M. W. Bruford, Institute of Zoology, Zoological Society of London, London, NW1 4RY, UK.

---

*To whom correspondence should be addressed. E-mail: tsmith@sfsu.edu tone-ecotone comparisons (r = 0.02 and 0.04, respectively, and $P > 0.10$ for Mantel's test). These data show that some ecotone populations are highly divergent morphologically despite substantial gene flow between them and populations in the central rainforest (Fig. 2). Because morphologic divergence and gene flow are significantly correlated only in ecotone-forest comparisons, where environmental variables differ, natural selection is likely the primary factor causing phenotypic divergence (*1*, *2*). If drift were responsible for morphological divergence, within-habitat comparisons would show an amount of morphological divergence per unit of gene flow that was similar to that seen in between-habitat comparisons, which is not the case. Furthermore, if genotype-environment interactions were responsible for morphological divergence, all ecotone-forest comparisons would show a similar degree of morphological divergence regardless of gene flow. Finally, geographic distance and gene flow are not significantly correlated in ecotone-ecotone, forest-ecotone, and forest-forest comparisons, which indicates an absence of isolation by distance. A correlation between the two variables would be expected if limited dispersal and drift were important causes of differentiation. Additionally, the finding of low values of morphologic divergence in ecotone-ecotone and forest-forest comparisons, despite a wide range in the values of *Nm*, suggests uniformity in the intensity and directionality of selection within ecotone and forest habitats.

Because rates of gene flow based on microsatellite data may be higher because of the influence of high mutation rates and the parallel evolution of same-size alleles (*17*), we also calculated *Nm* on the basis of a limited study of mitochondrial control region sequences. We assessed variability in 293 base pairs of region 1 of the avian control region using published primers LGL2 and H417 (*18*). We sampled a total of 64 individuals from three ecotone and four central rainforest populations (including Wakwa, Bétaré Oya, Tibati, Ndibi, Nkwouak, Zoebefame, and Kribi) (see Fig. 1). Results indicate high gene flow between populations; the average pairwise value of *Nm* was 1.1 migrants per generation, which is less than the value of 5.1 based on microsatellite data but of the same magnitude. Moreover, the sequence divergence between populations was low but genotype diversity was high, suggesting recent population expansion. We found 25 sequences in 64 individuals; the average sequence divergence between forest localities was 1.1%, between forest and ecotone localities it was 0.95%, and between ecotone localities it was 0.82%.

Laboratory experiments involving *Drosophila* have shown that intense directional selection on morphologic traits in the presence of gene flow often results in prezygotic isolation (*4*). These studies support the divergence-with-gene-flow model of speciation, which asserts that divergent selection, through linkage between reproductive traits and those traits under selection, results in speciation (*4*). Although we do not demonstrate evidence of reproductive isolation between populations of *A. virens*, the magnitude of morphological differences between ecotone and forest populations is as large as that found between sympatric congeneric species (Fig. 3) (*19*). Consequently, our data support a central component of the divergence-with-gene-flow model of speciation by suggesting that despite large amounts of gene flow, selection is sufficiently intense to cause morphologic differences similar to those separating reproductively isolated species. The divergence-with-gene-flow model of speciation predicts, using the same logic that has been used for classical allopatric speciation, that such persistent divergent selection may lead to reproductive isolation, but here the selective differences accelerate the process.

In conclusion, we suggest that ecotones, when geographically large, are habitats in which populations may diverge from their rainforest counterparts. The generality of our results is supported by patterns of morphological divergence documented for many avian species, showing that major contact zones between species and subspecies are concentrated in ecotones (*8*–*10*), and it supports previous assertions that eco-

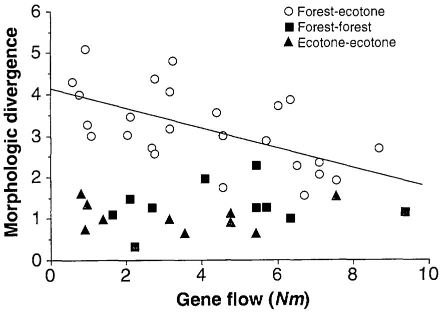

Fig. 2. The relation between *Nm* and multivariate morphologic divergence. Open circles represent pairwise comparisons between populations in forest patches occurring in the ecotones and contiguous forest populations, solid triangles represent populations occurring in the forest patches within the ecotone, and solid squares represent populations occurring in the contiguous rainforest (see Fig. 1; sites 2 and 12 were excluded because of low sample sizes).

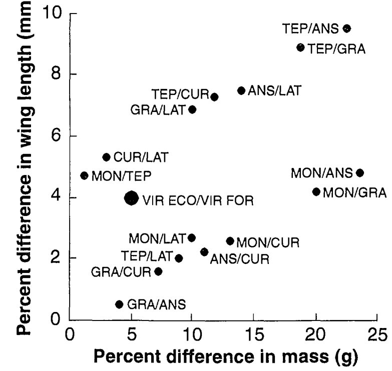

Fig. 3. Differences in wing length and body mass between sympatric congeneric species of *Andropadus gracilis* (GRA), *A. ansorgei* (ANS), *A. curvirostris* (CUR), *A. latirostris* (LAT), *A. montanus* (MON), *A. tephrolaemus* (TEP), and the ecotone and forest populations of *A. virens* (VIRECO/VIRFOR). Character means for *A. virens* are from Table 1, and those for the other species are from (*22*); all are for adults, with the sexes combined.

Table 1. Character sizes of adult *A. virens* from forest and ecotone sites in Cameroon. Characters that differ significantly between forest sites include weight and wing length ($P < 0.01$, multivariate analysis of variance); between ecotone sites, only tarsus length differs significantly ($P < 0.001$). All measurements were taken by Thomas B. Smith with dial calipers except mass, which was measured with a 50-g Pesola spring scale. The measurements were taken as follows: wing length, from the carpal joint to the tip of the longest primary; tarsus length, from the tibiotarsal joint to the distal undivided scute; upper mandible length, the chord length from the point where the culmen enters the feathers of the head to the tip; bill depth, in the vertical plane level at the anterior edge of the nares. Juveniles that were distinguishable from adults on the basis of their yellow gape were excluded; however, because males and females are indistinguishable, the sexes were combined for analysis.

Character	Forest		Ecotone		df	*F*	*P*
	n	Mean ± SE	*n*	Mean ± SE			
Weight (g)	45	22.4 ± 0.399	60	24.8 ± 0.335	102	21.9	<0.001
Wing length (mm)	45	70.9 ± 0.453	61	76.8 ± 0.468	102	84.57	<0.001
Tarsus length (mm)	45	19.2 ± 0.126	61	20.2 ± 0.112	102	31.19	<0.001
Upper mandible length (mm)	45	12.1 ± 0.121	61	11.9 ± 0.121	102	1.26	0.265
Bill depth (mm)	44	4.2 ± 0.037	61	4.3 ± 0.033	102	6.31	0.014
Multivariate Wilk's lambda					99	20.53	<0.001

tones are potentially important regions of differentiation and speciation that conceivably may enrich the biodiversity of tropical rainforests (*9*). Three observations lead to this conclusion. First, as we have shown for the little greenbul, divergent selection rather than drift or other factors has most likely caused morphologic differentiation in ecotone populations. Second, the magnitude of divergence in fitness-related characters is similar to that found between reproductively isolated species, which suggests that selection differences are large between ecotone and forest habitats. Third, laboratory experiments suggest that such divergent selection may sometimes lead to reproductive divergence. Our results suggest that ecotones may be integral to the production and maintenance of biodiversity in tropical rainforests. This contrasts with past theories of rainforest speciation that have focused on within-rainforest vicariance mechanisms (*20*). Currently, because ecotones may exhibit less species richness than central rainforest habitats, comparatively less attention is paid to conserving them. If further research supports the role of ecotones as centers for speciation, their dynamics will need to be preserved so that rainforest biodiversity can be sustained and replenished.

REFERENCES AND NOTES

1. M. Slatkin, *Science* **236**, 787 (1987).
2. J. A. Endler, *Geographic Variation, Speciation, and Clines*, (Monogr. Popul. Biol. 10, Princeton Univ. Press, Princeton, NJ, 1977).
3. A. R. Templeton, *Evolution* **41**, 233 (1987); E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, MA, 1963).
4. R. R. Rice and E. E. Hostert, *Evolution* **47**, 1637 (1993).
5. A. C. Millington, P. J. Styles, R. W. Critchley, in *Nature and Dynamics of Forest-Savanna Boundaries*, P. A. Furley, J. Proctor, J. A. Ratter, Eds. (Chapman and Hall, New York, 1992), pp. 37–62.
6. K. A. Longman and J. Jenik, *ibid.*, pp. 3–20.
7. T. B. Smith, *Nature* **363**, 618 (1993).
8. J. P. Chapin, *Bull. Am. Mus. Nat. Hist.* **75**, 1 (1954).
9. J. A. Endler, *Am. Zool.* **22**, 441 (1982); in *Biological Diversification in the Tropics*, G. T. Prance, Ed. (Columbia Univ. Press, New York, 1982), pp. 641–657.
10. B. P. Hall and R. E. Moreau, *An Atlas of Speciation in African Passerine Birds* (British Museum, London, 1970); T. M. Crowe and A. A. Crowe, *J. Zool. London* **198**, 417 (1982); J. P. Chapin, *Bull. Am. Mus. Nat. Hist.* **65**, 1 (1932).
11. The number, location, and dates of field work for each forest site were as follows: 1, Kribi (2°43′N, 9°52′E), 6 to 9 October 1990 and 1 to 4 July 1993; 2, Ndibi (3°46′N, 12°12′E), 28 July to 20 October 1990; 3, Zoebefame (2°39′N, 13°23′E), 27 to 29 September 1990 and 9 to 15 June 1993; 4, Bobo Camp (2°39′N, 13°28′E) 15 to 20 June 1993; 5, Nkwouak (3°52′N, 13°18′E), 7 to 8 August 1990 and 29 June to 2 July 1993; and 6, Lac Lobeke (2°18′N, 15°45′E), 25 to 29 June 1993. Those for each ecotone site were as follows: 7, Tibati (6°30′N, 12°35′E), 26 to 29 August 1990 and 5 to 10 July 1995; 8, Ngaoundaba Ranch (7°08′N, 13°41′E), 19 to 23 May 1995; 9, Wakwa, (7°16′N, 13°31′E), 21 to 23 August 1990 and 29 June to 4 July 1995; 10, Bétaré Oya (5°34′N, 14°05′E), 11 to 13 August 1990 and 5 to 8 May 1995; 11, Meiganga (6°31′N, 14°18′E), 15 to 19 August 1990 and 10 to 14 May 1995; and 12, Djohong (6°50′N, 14°42′E), 15 to 17 May 1995.
12. P. T. Boag and A. van Noordwijk, in *Avian Genetics: A Population and Ecological Approach*, F. Cooke and P. A. Buckley, Eds. (Academic Press, New York, 1987), pp. 45–78.
13. B. R. Grant and P. R. Grant, *Proc. R. Soc. London Ser. B Biol. Sci.* **251**, 111 (1993); T. B. Smith, *Evolution* **44**, 832 (1990); D. Schluter and J. N. M. Smith, *ibid.* **40**, 221 (1986); D. Schluter, *Ecol. Monogr.* **58**, 229 (1988).
14. C. W. Benkman, *Evol. Ecol.* **5**, 118 (1991).
15. S. Wright, *Evolution and the Genetics of Populations* (Univ. of Chicago Press, Chicago, IL, 1968), vol. 1.
16. M. Nei, *Molecular Evolutionary Genetics* (Columbia Univ. Press, New York, 1987).
17. D. B. Goldstein, A. Ruiz Linares, L. L. Cavalli-Sforza, M. W. Feldman, *Genetics* **139**, 463 (1995); M. Slatkin, *ibid.*, p. 457.
18. C. L. Tarr, *Mol. Ecol.* **4**, 527 (1995).
19. Ecotone-forest differences are greater than a third of the interspecific differences in wing length and a fifth of those in mass. When between-species differences in tarsus length, upper mandible length, and weight were contrasted by means of principal components analysis, the results were similar. Using the difference in mean character size between species and between ecotone and forest populations of *A. virens*, we calculated principal components using a correlation matrix. Forest-ecotone comparisons of *A. virens* were greater than 2 and 11 of the interspecific comparisons, along PC1 and PC2, respectively.
20. E. Mayr and R. J. O'Hara, *Evolution* **40**, 55 (1986); J. Haffer, *Science* **165**, 131 (1969).
21. R. Letouzey, *Etude Phytogeographique du Cameroun*, vol. 69 of *Encyclopedie Biologie* (Lechevalier, Paris, 1968).
22. S. Keith, E. K. Urban, C. H. Fry, *The Birds of Africa* (Academic Press, New York, 1992), vol. 4.
23. We thank the government of the Republic of Cameroon for permission to conduct the field research; J. Endler, M. Kirkpatrick, S. MacLane, C. Mortiz, M. Orr, S. Palumbi, E. Routman, C. Schneider, M. Slatkin, M. Turelli, B. Van Valkenburgh, and J. Carothers for helpful discussion and comments; and B. Larison, D. McNiven, R. Fotso, K. Whitney, M. Kimura, M. Fogiel, T. Robson, R. Zebell, and E. Taylor for assistance with field and laboratory work. The research was supported by the National Geographic Society, NERC grant GR3/8551′A′, the World Wildlife Fund, Royal Society grant 571310.V703, and NSF grant BSR 88-17336.

27 January 1997; accepted 18 April 1997

Macrophages as a Source of HIV During Opportunistic Infections

Jan M. Orenstein,* Cecil Fox, Sharon M. Wahl

The source of increasing viremia that characterizes the latter stages of human immunodeficiency virus (HIV) disease has remained a paradox because it occurs at a time when lymphoid tissue is quantitatively and qualitatively impaired, and the patients' CD4 T lymphocytes are steadily declining. Here, macrophages, both infected and uninfected with common opportunistic pathogens of HIV disease such as *Mycobacterium avium* complex and *Pneumocystis carinii*, were identified as highly productive sources of HIV in coinfected lymph nodes. These observations indicate that tissue macrophages are not only infected with HIV, but that common pathogens of HIV disease can dramatically increase their production of virus. Thus, prevention or successful treatment of opportunistic coinfections, or both, potentially benefits the patient twofold by limiting the pathology caused by opportunistic infection and by controlling induction of HIV replication.

Opportunistic infections (OIs) and rising levels of circulating human immunodeficiency virus–type 1 (HIV-1) are hallmarks of progressing HIV-1 disease (*1*). After infection with HIV-1, a variably paced loss of CD4 T lymphocytes leads to immunodeficiency and increasing susceptibility to a broad range of opportunistic viral, bacterial, fungal, and protozoal pathogens. A direct correlation between OIs and HIV-1 expression in vitro is paralleled by emerging in vivo evidence (*2*). Infections with common opportunistic pathogens, such as herpesvirus type-1, *Mycobacterium avium* complex, and *M. tuberculosis* are accompanied by mostly reversible increases in HIV-1 viremia (*2–4*). Bacterial pneumonia, an increasing cause of morbidity and mortality in HIV-1 infection, is also associated with increased circulating HIV-1 (*5*).

The source of the increased circulating HIV-1 has remained a puzzle because progression of HIV-1 disease is also characterized by involution of lymphoid tissue. Lymph nodes characteristically display progressive loss of germinal centers, leaving little if any remaining follicular dendritic cell–associated virus and only scattered productively infected CD4$^+$ lymphocytes. The role of the long-lived, mobile phagocyte as a source of virus during HIV-1 disease has been considered minimal, as few if any productively infected cells expressing monocyte/macrophage

---

J. M. Orenstein, Department of Pathology, George Washington University, Washington, DC 20037, USA.
C. Fox, Molecular Histology Inc., Gaithersburg, MD 20879, USA.
S. M. Wahl, Oral Infection and Immunity Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892, USA.

*To whom correspondence should be addressed. E-mail: jmo@gwis2.circ.gwu.edu