that it caused depolarization of somatotrophs, whereas somatostatin caused hyperpolarization. The bioassay of L-692,429 has been evaluated in more than 50 receptor binding assays and more than 20 functional assays in which known ligands stimulate a biochemical or biological response. With the exception of weak angiotensin II receptor binding (concentration required for 50% inhibition was 6 μM), L-692,429 is inactive at <10 μM.

Selective nonpeptidyl kappa receptor agonists are known (22), although few other examples of potent and specific nonpeptidyl mimetic agonists and antagonists of peptide ligands exist (23). Structural modifications of L-692,429 and establishment of a structure-activity relationship as agonists or antagonists provide new and important clues to the design of nonpeptidyl mimics of peptides. By the substitution of pharmacophores, structural elements that elicit bioactivity, small molecules can be tailored to fit the receptor sites of other peptides. The advantage of such mimetics is that their structures can be readily modified either subtly or dramatically to provide the structural diversity necessary to optimize molecules suitable as oral drugs.

REFERENCES AND NOTES

24. After 3 days in cultures, rat pituitary cells were dispersed with trypsin (0.25 mg/ml) and GH-secreting cells were identified by a reverse hemo- lytic plaque assay (17). The cells attached to a glass cover slip were loaded with 1.5 μM fura-2/AM (Calbiochem) for 30 min at 37°C in 0.1% BSA-DMEM (bovine serum albumin–Dubcco’s modified Eagle’s medium). The cells were then rinsed with Hanks’ solution containing 10 mM Hepes (pH 7.4) and 0.1% BSA and were given 5 min to allow fura-2/AM de-esterification. The glass cover slip was then placed in a Micro-Cellubator with a temperature control set at 37°C (Medical Sys- tem Corp., Greenvale, NY) and placed on the Nikon microscope stage of a Deltasonic 4000-P2 ratio imaging system equipped with dual excita- tion monochromators (Proton Technology Interna- tional, South Brunswick, NJ). The fluorescence emission of a somatotroph was monitored at 510 nm with a Hamamatsu intensified charged-cou- pled device camera. The cytotoxic-free Ca²⁺ concentration was measured by the ratio of fluo- rescence excited by 340 nm to that excited by 380 nm and was calibrated with the equation developed by G. Brykiewicz, M. Poenie, and R. Y. Tsein (J. Biol. Chem. 260, 3440 (1985)). Values for R_red/R_blue (where R is the fluorescence ratio) were obtained from measurements with a solution of 20 mM fura-2 and 10 mM EGTA. R_red was measured after addition of 20 μM ionomycin. The R_max/R_red value was approximately 30. The results shown were obtained with a solution of L-692,429 that caused a maximal release of GHR (2 μM). Of nine somatotrophs tested, eight responded to L-692,429 with the concentration of Ca²⁺ increasing from 102 ± 34 (SE) to 516 ± 274 nM; no increase was evident with L-692,428.
25. The Ω Agatkov ΩIIa is a gift from M. Smith (Merck Research Laboratories).
11 December 1992; accepted 8 March 1993

Unidirectional Spread of Secondary Sexual Plumage Traits Across an Avian Hybrid Zone

Thomas J. Parsons,†* Storrs L. Olson, Michael J. Braun

Theory predicts that traits under positive selection can rapidly cross a hybrid zone in spite of a substantial barrier to neutral gene flow between hybridizing taxa. An avian hybrid zone between Manacus candei (white-collared manakin) and M. vitellinus (golden-collared manakin) is reported here that displays an unusual pattern of noncoincident clines. Male secondary sexual traits of M. vitellinus have spread into populations that are genetically and morphometrically like M. candei. These birds have a lek breeding system in which male mating success is highly skewed, suggesting that sexual selection is driving male sexual traits across the zone.

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flows into the Río Changuinola, which forms a substantial discontinuity in the forest habitat. At locality 1, we found only golden-colored birds, similar to birds designated as *M. vitellinus cinnius* (7). At localities 8 and 9, we found *M. vitellinus vitellinus*, whose range continues eastward throughout Panama. Birds from localities 4 through 7 show plumage characteristics that are both intermediate between those of *M. candei* and *M. v. vitellinus* and markedly variable. No pure specimens of these taxa were found in this region (Fig. 2 and Table 1).

Males of *M. v. vitellinus* have a narrow orange-golden collar and throat, a small patch of yellow on the bend of the wing, and olive-green underparts. Males of *M. candei* have a broad white collar and throat, an extensive white patch on the bend of the wing, and bright yellow underparts. Males of *M. cinnius* have plumage characteristics intermediate between those of *M. candei* and *M. v. vitellinus*, with an intermediate-width, lemon-yellow collar, intermediate-sized wing patch, and yellow-green underparts. In addition to being variable, birds from localities 4 through 7 show clinal patterns, on average, for three male plumage traits (throat color, collar width, and color of underparts) (Fig. 2 and Table 1). Birds from localities 4 through 7 become more similar to *M. v. vitellinus* as one approaches locality 8, where phenotypically pure *M. vitellinus* is found. The intermediacy, variability, and clinal variation of male plumage traits of birds from localities 4 through 7 are indicative of introgressive hybridization between *M. candei* and *M. v. vitellinus* (8).

Further evidence for hybridization between *M. candei* and *M. vitellinus* was obtained from an analysis of mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs). Restriction fragment patterns diagnostic of *M. candei* (locality 1) and *M. vitellinus* (locality 10) were found with 6 of 13 enzymes tested (9). The "candei" mtDNA haplotype was found in all *M. candei*, which have white collars, and in all birds from localities 4 through 7, which have golden collars (Fig. 2 and Table 2) (10). The *M. v. vitellinus*-like birds sampled at localities 8 through 10 had primarily the "vitellinus" haplotype, although evidence of introgression was seen: 1 of 20 birds from locality 8 and 2 of 20 from locality 9 had the candei haplotype. A discrete break in haplotype frequencies occurs between localities 7 and 8, which does not coincide with the shift from white- to golden-collared traits at the Río Changuinola between localities 3 and 4.

The lack of concordance between the clines for plumage color traits and mtDNA haplotype may be due to differential introgression of either the *M. candei* mtDNA haplotype (11) or the *M. vitellinus* male plumage traits. Differential introgression of the male plumage traits may be driven by intense sexual selection at *Manacus* leks.

To examine these possibilities, we measured eight morphometric characters from birds across the region. Two of these, tail length and beard length, distinguished the parental populations (12). Both characters shift between localities 7 and 8, matching the shift of mtDNA haplotype but not the shift from white to golden collars that occurs between localities 3 and 4 (Fig. 2 and Table 1).

Independent RFLPs were obtained with random nuclear DNA probes. Two enzyme-probe combinations distinguished the parental populations (13). The *M. vitellinus* parental sample was fixed for the "vitellinus" allele in both markers, but the *M. candei* parental sample had a "candei" allele frequency of 92% for both markers. Shifts in allele frequency of both nuclear DNA markers coincide with the shifts of mtDNA haplotype and morphometric markers, with an abrupt break between localities 7 and 8 (Fig. 2). *Manacus candei* alleles were found in a few birds from localities 8 and 9, confirming hybridization and limited eastward introgression of these characters.

The abrupt shifts in mtDNA haplotype, two morphometric markers, and two ran-

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**Table 1. Sample size (n), population mean (x̄), and SD for male morphological characters across the region. Locality numbers are as in Fig. 1. Color indices are as described in (20). Sample sizes differ among characters depending on the type of specimen preparation. (**) Collar width measurements are affected by the style of study skin preparation.**

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**Fig. 1. Distribution of *Manacus* and sampling localities on the mainland of Bocas del Toro Province, Panama. (Inset) Isthmus of Panama, showing region of the expanded map. Hatched regions, ranges of three *Manacus* forms with distributions restricted to a narrow strip of lowland forest along the coast. Question marks, lowland areas where specimens of *Manacus* have not been obtained. Localities: 1, Guápiles, Costa Rica (140 km northwest of mapped region); 2, Rio Siquia; 3, Rio Tenere; 4, Rio Changuinola; 5, Rio Oeste; 6, Quebrada Pastores; 7, Tierra Oscura; 8, Chiriqui Grande; 9, Valiente Peninsula; and 10, Panama Canal (250 km east of mapped region).**
Table 2. Sample size (n) and allele or haplotype frequency (f) for candei genetic markers across the region. Locality numbers are as in Fig. 1. Sample sizes are larger than for morphological data (Table 1) owing to the inclusion of data from female and male-plumaged birds.  

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<td>3</td>
<td>0.83</td>
<td>20</td>
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<td>20</td>
<td>0.83</td>
<td>21</td>
<td>0.94</td>
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<td>0.83</td>
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dom nuclear DNA markers indicate that a barrier to gene flow exists between localities 7 and 8. Birds sampled from localities 4 through 7 are genetically and morphometrically indistinguishable from M. candei (14), although their plumage characteristics resulted in their classification as a subspecies of M. vitellinus. Alleles for golden-collared plumage characteristics apparently have introgressed some 40 km to the west of the barrier between localities 7 and 8, ending at the Rio Changuinola (15).  

Mating success at Manacus leks is highly skewed: in one lek of twenty males, one male received 73% of all observed copulations, and this male and five others received 95% of all observed copulations (16). We hypothesize that such a strongly mating bias is driving the unidirectional introgression of golden-collared plumage traits across the hybrid zone (17, 18). Introgression of only part of the genome might have occurred because most hybrid genotypes are selected against, and alleles for positively selected plumage traits have spread after recombining into a candei genetic background.  

Our data reflect a single point in time; the boundary between white and golden collars may yet be moving westward (19). The data suggest that positive sexual selection is driving reproductively advantageous traits across a barrier to gene flow in these natural populations.

REFERENCES AND NOTES

3. For discussion of alternative mechanisms of sele-
ction see W. S. Moore (J. Exp. Biol. 62, 263 (1977)).
5. Manacus sexual behavior has been studied pri-
marily in M. manacus (white-bearded manakin) [D. W. Snow, Zoologica 47, 65 (1962); A. Lill, Z. Tierpsychol. 36, 36 (1974); ibid., p. 513]. The behavior of M. vitellinus is similar in virtually all respects [F. M. Chapman, Bull. Am. Mus. Nat. Hist. 68, 471 (1935)]; the behavior of M. candei, although generally similar, has not been studied in detail.
6. Expeditions by the Smithsonian Institution were conducted during dry-season months (February through April) from 1987 to 1991. The region was found to be of great biogeographic interest and complexity [S. L. Olson, Auk 110, 100 (1993)]. The islands of the region harbor additional golden-collared forms of Manacus, likely of hybrid origin.
7. Manacus cernita was designated as a species from studies of eight birds collected near the locality 5 [J. L. Peters, Proc. N. Engl. Zool. Club 10, 9 (1927)], but is currently classified as a subspe-
cies of M. vitellinus (American Ornithologists’ Union, Check-list of North American Birds (Aiken Press, Lawrence, KS, ed. 6, 1983)).
8. The possible hybrid nature of M. v. cernita-type birds was suggested by E. Eibesmann (J. Helfer, Am. Mus. Novit. 2294, 1 (1967)) and would likely have been generally recognized were it not that M. candei was only recently discovered in Para-
mania (R. S. Rigby and J. A. Gwynne, Jr., A Guide to the Birds of Panama with Costa Rica, Nicaragua, and Honduras (Princeton Univ. Press, Prin-
ceton, NJ, ed. 2, 1969)).
9. DNA was digested with restriction enzymes, elec-
trophoresed in agarose gels, and Southern (DNA) blotted to nylon filters with the use of similar meth-
ods (J. Sambrook, E. F. Fritsch, T. Maniatis, Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989)). Immobilized DNA was hybridized with radiolabeled, purified mitochondrial DNA from M. vitellinus or house finch (Carpodacus mexicanus). When C. mexicanus mtDNA was used as probe, the Southern blots were washed less stringently to yield patterns equivalent to those obtained with the manakin probe. The estimated mtDNA sequence diver-
gence, p = 1.4% (M. Nel and W.-H. Li, Proc. Natl. Acad. Sci. U.S.A. 76, 5269 (1979)), is a value
Evidence of DNA Bending in Transcription Complexes Imaged by Scanning Force Microscopy

William A. Rees, Rebecca W. Keller,* James P. Vesenka,† Guoliang Yang, Carlos Bustamante‡

Complexes of Escherichia coli RNA polymerase with DNA containing the $\Lambda P_L$ promoter have been deposited on mica and imaged in air with a scanning force microscope. The topographic images reveal the gross spatial relations of the polymerase relative to the DNA template. The DNA appears as a double-exponent promoter complex bound to the promoter and appears more severely bent in elongation complexes in which RNA polymerase has synthesized a 15-nucleotide transcript. This difference could be related to the conformational changes that accompany the maturation of open promoter complexes into elongation complexes and suggests that formation of the elongation complex involves a considerable modification of the spatial relations between the polymerase and the DNA template.

Scanning force microscopy (SFM) has been used to generate high-resolution images of nucleic acids (1-7) and proteins (8, 9). Some of these images were obtained under conditions that preserve the state of hydration of the macromolecules, which suggests that SFM could be a powerful tool for investigation of the structure of complex macromolecular assemblies in their native conformation (10). This report describes the homogeneous deposition and reliable imaging of complexes of E. coli RNA polymerase and a DNA fragment containing the $\Lambda P_L$ promoter. In particular, we investigated the spatial relations between the RNA polymerase and the DNA template in open promoter complexes (OPCs) and in stable elongation complexes with a nascent 15-nucleotide transcript (C15 complexes).

Transcription complexes were prepared by a modification of the method of Levin et al. (11) and made up of a 681-base pair (bp) DNA fragment as the template (12). Complexes were imaged at low humidity in air with a Nanoscope II SFM (Digital Instruments) with tips that were modified with an electron beam (1, 2, 13). Samples were prepared in a low-salt buffer containing 5 mM MgCl2 and deposited onto freshly cleaved, previously unmodified mica. The remainder of the deposition procedure was based on a previously published method (1). Images were obtained within 30 min of sample preparation.

Unbound DNA, unbound RNA polymerase, and RNA polymerase–DNA complexes can be seen against a relatively flat background in representative fields of complexes (Fig. 1). The DNA templates with RNA polymerase located about four-ninths of the distance from one end (at the $\Lambda P_L$ promoter) were identified as complexes. In images of individual OPCs (Fig. 2A) and C15 complexes (Fig. 2B), the RNA polymerase appears as a slightly asymmetric structure situated astride the template with the flanking arms of the DNA meeting underneath the polymerase.

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