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

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that it caused depolarization of somatotrophs, whereas somatostatin caused hyperpolarization. The specificity 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.

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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 hemolytic 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–Dulbecco'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 mounted on a Micro-Incubator with a temperature control set at 37°C (Medical System Corp., Greenvale, NY) and placed on the Nikon microscopic stage of a Deltascan 4000-52 ratio imaging system equipped with dual excitation monochromators (Proton Technology International, South Brunswick, NJ). The fluorescence emission of a somatotroph was monitored at 510 nm with a Hamamatsu intensified charged-coupled device camera. The cytosolic free Ca^{2+} concentration was measured by the ratio of fluorescence excited by 340 nm to that excited by 380 nm and was calibrated with the equation developed by G. Grynkiewicz, M. Poenie, and R. Y. Tsein [*J. Biol. Chem.* **260**, 3440 (1985)]. Values for R_{min} (where R is the fluorescence ratio) were obtained from measurements with a solution of 20 μM fura-2 and 10 mM EGTA. R_{max} was measured after addition of 20 μM ionomycin. The R_{max}/R_{min} value was approximately 30. The results shown were obtained with a concentration of L-692,429 that caused a maximal release of GH (2 μM). Of nine somatotrophs tested, eight responded to L-692,429 with the concentration of Ca^{2+} increasing from 102 ± 34 (SE) to 516 ± 274 nM; no increase was evident with L-692,428.
25. The Ω Agatoxin IIIA is a gift from M. Smith (Merck Research Laboratories).

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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.

Hybrid zones provide insight into evolutionary forces that subdivide populations and species and influence the spread of alternative adaptations (*1*). The tension zone model proposes that stability of narrow hybrid zones is maintained through a balance between selection against hybrids and dispersal of parental types across range boundaries (*2*). Selection may act against hybrid individuals because they have disadvantageous combinations of coadapted gene complexes from each parental taxon (*1*, *3*). Such selection could create a barrier to gene flow for neutral alleles linked to negatively selected loci and produce the commonly observed pattern of narrow, concordant shifts in diagnostic characters across hybrid zones. However, mathematical models predict that alleles under positive selection can spread rapidly across hybrid zones (*4*). Thus, hybrid zones may serve as evolutionary conduits between locally adapted populations, through which generally advantageous traits can spread despite a barrier to overall gene flow across the zone.

Birds in the genus *Manacus* are small, frugivorous inhabitants of lowland tropical rainforest from Mexico to Argentina. Highly sexually dimorphic, they have a lek breeding system in which the brightly colored males display at court assemblages nearly year round. The olive-colored females visit a lek to select a mate, then take sole responsibility for nesting and raising the young. Males compete for females by performing courtship displays. Female mate choice is nonrandom, few males receiving most of all copulations (*5*).

Males of *Manacus* have a black cap contrasting with a bright collar, throat, and feather beard. The species differ most obviously in the color of the throat, collar, and underparts. Male courtship display accentuates the contrast between the cap and collar by thrusting of the head and neck forward and extending of the beard.

When surveyed in Bocas del Toro Province, Panama (*6*), white-collared birds typical of M. *candei* were found only in northwestern Bocas del Toro at localities 2 and 3 (Fig. 1). Near locality 3, the Río Teribe

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flows into the Río Changuinola, which forms a substantial discontinuity in the forest habitat. At locality 4, we found only golden-collared birds, similar to birds designated as *M. vitellinus cerritus* (*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. v. cerritus* 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. v. 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-

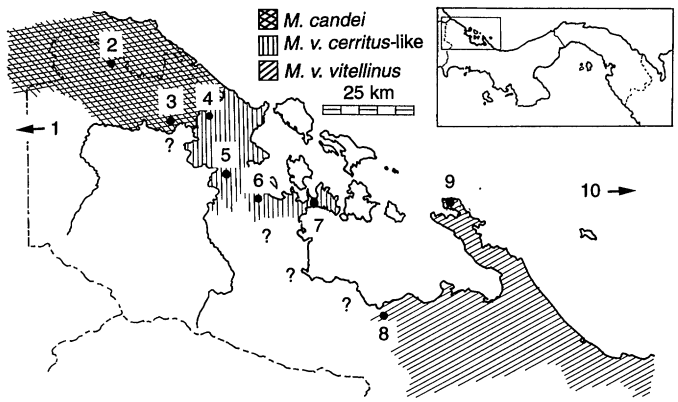

Fig. 1. Distribution of *Manacus* and sampling localities on the mainland of Bocas del Toro Province, Panama. (Inset) Isthmus of Panama, showing region of the expanded map. Hatched regions, ranges of three *Manacus* forms with distributions restricted to a narrow strip of lowland forest along the coast. Question marks, lowland areas where specimens of *Manacus* have not been obtained. Localities: 1, Guápiles, Costa Rica (140 km northwest of mapped region); 2, Río Sixaola; 3, Río Teribe; 4, Río Changuinola; 5, Río Oeste; 6, Quebrada Pastores; 7, Tierra Oscura; 8, Chiriquí Grande; 9, Valiente Peninsula; and 10, Panama Canal (250 km east of mapped region).

Table 1. Sample size (*n*), population mean ($\bar{x}$), 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. Study skins from localities 2 through 10 were prepared by Smithsonian expedition members in a consistent style. Collar width data from locality 1 are not included because these specimens (from a 1920s collection in the Carnegie Museum of Natural History) were prepared in a more compact style.

Locality	1			2			3			4			5			6			7			8			9			10		
	n	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD
Beard length (cm)	19	1.17	0.07	3	1.21	0.03	9	1.18	0.08	9	1.20	0.08	16	1.18	0.12	5	1.22	0.02	11	1.19	0.16	11	1.60	0.16	5	1.64	0.19	20	1.66	0.15
Tail length (cm)	19	3.42	0.08	3	3.44	0.12	17	3.41	0.09	13	3.44	0.11	16	3.47	0.13	5	3.44	0.06	11	3.39	0.16	11	2.84	0.13	5	2.88	0.08	20	2.84	0.08
Throat color	19	6.0	0.00	3	6.0	0.00	17	6.0	0.00	9	4.1	0.53	16	3.9	0.66	5	3.5	0.50	11	3.2	0.68	11	2.2	0.60	5	1.9	0.74	20	1.4	0.43
Underparts color	19	5.0	0.00	3	5.0	0.00	17	5.0	0.00	9	3.9	0.85	16	3.1	0.89	5	2.2	0.57	11	1.8	0.60	11	1.1	0.09	5	1.0	0.00	20	1.0	0.03
Collar width (cm)		***		3	2.4	0.23	17	2.4	0.24	13	2.2	0.29	16	2.0	0.25	5	1.9	0.43	11	1.6	0.39	11	0.9	0.18	5	0.9	0.40	20	0.7	0.18

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 immature-plumaged birds.

Locality	1		2		3		4		5		6		7		8		9		10	
	n	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*
mtDNA	6	1.00	3	1.00	20	1.00	20	1.00	21	1.00	4	1.00	12	1.00	20	0.05	20	0.10	20	0.00
λ5	6	0.92	3	0.83	20	1.00	20	0.83	21	0.95	4	1.00	12	0.88	20	0.12	20	0.00	20	0.00
pSCN3	6	0.92	3	0.67	20	0.83	20	0.83	21	0.83	4	0.88	12	0.83	20	0.08	20	0.03	20	0.00

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 Río 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 strong 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.

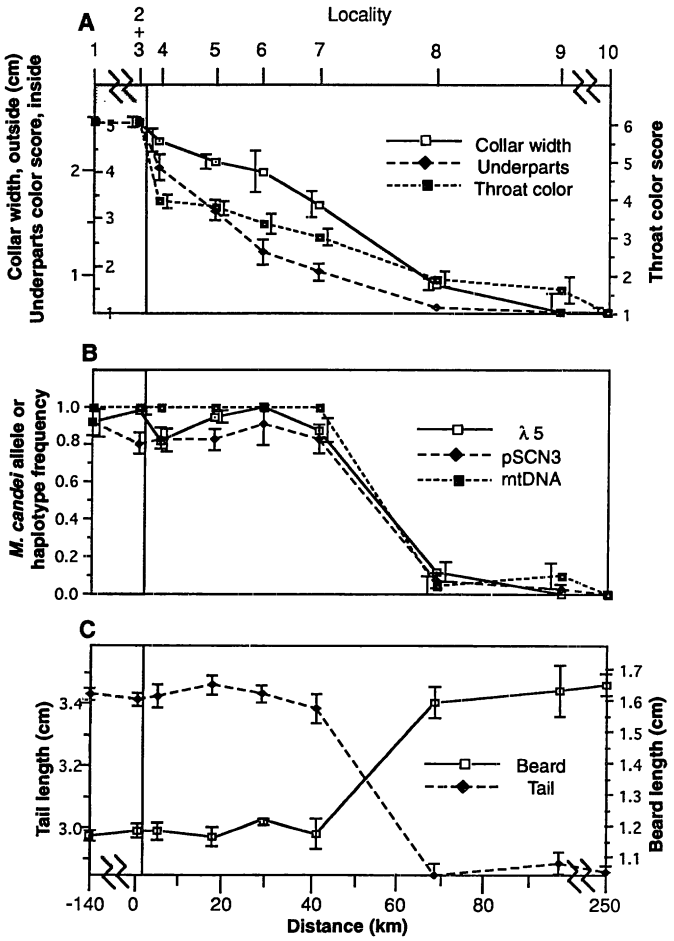

Fig. 2. Population means and allele or haplotype frequencies of diagnostic characters across the zone; *x* axis, distance along a northwest to southeast line. Localities are as in Fig. 1. Localities 1 and 10 lie off the scale. Distance to locality 9 is measured to the base of the Valiente Peninsula. Owing to small sample size at locality 2, these birds are merged with those of locality 3 to represent all *M. candei* birds from Panama (Tables 1 and 2). All birds in the shaded portion of the graph have white collars typical of *M. candei*, whereas all birds in the unshaded portion have golden collars. The shift from white to golden collars occurs at least 40 km west of the shift in genetic markers (B) and the shift in morphometric markers (C). Error bars are standard errors; standard deviations are given in Table 1. (**A**) Clines for male plumage characters. Left-outside *y* axis (in centimeters), collar width; left-inside *y* axis, underparts index score; and right-outside *y* axis, throat color index score (*20*). Standard error bars for underparts are centered on the data point, those for collar width are displaced to the left, and those for throat color are displaced to the right. (**B**) Clines for three genetic markers. Standard error bars for pSCN3 are centered, those for mtDNA are displaced to the left, and those for λ5 are displaced to the right. (**C**) Clines for morphometric markers.

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3. For discussion of alternative mechanisms of selection see W. S. Moore [*Q. Rev. Biol.* **52**, 263 (1977)].
4. N. H. Barton, *Heredity* **43**, 333 (1979).
5. *Manacus* sexual behavior has been studied primarily 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 cerritus* was designated as a species from studies of eight birds collected near locality 5 [J. L. Peters, *Proc. N. Engl. Zool. Club* **10**, 9 (1927)], but is currently classified as a subspecies of *M. vitellinus* [American Ornithologists' Union, *Check-list of North American Birds* (Allen Press, Lawrence, KS, ed. 6, 1983)].
8. The possible hybrid nature of *M. v. cerritus*–type birds was suggested by E. Eisenmann [J. Haffer, *Am. Mus. Novit.* **2294**, 1 (1967)] and would likely have been generally recognized were it not that *M. candei* was only recently discovered in Panama [R. S. Ridgely and J. A. Gwynne, Jr., *A Guide to the Birds of Panama with Costa Rica, Nicaragua, and Honduras* (Princeton Univ. Press, Princeton, NJ, ed. 2, 1989)].
9. DNA was digested with restriction enzymes, electrophoresed in agarose gels, and Southern (DNA) blotted to nylon filters with the use of standard methods (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 divergence, $p = 1.4\%$ [M. Nei and W.-H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5269 (1979)], is a value

typical for closely related avian congeners [J. C. Avise and R. M. Zink, *Auk* **105**, 516 (1988)]. The diagnostic enzymes Eco RV, Bgl II, and Ava I were selected for analysis of all individuals from each population across the region.
10. When DNA from all birds was analyzed with Bgl II, Ava I, and Eco RV, a few showed minor length variations in a single ~5-kb Eco RV band, but the haplotypes of all birds could be clearly related to one or the other parental type.
11. W. S. Moore, J. H. Graham, J. T. Price, *Mol. Biol. Evol.* **8**, 327 (1991); S. D. Ferris *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2290 (1983); N. Barton and J. S. Jones, *Nature* **306**, 317 (1983); J. R. Powell, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 492 (1983).
12. Tail length is not sexually dimorphic in these taxa, but only male birds have beards.
13. Using an approach similar to that of T. W. Quinn and B. N. White [in *Avian Genetics*, F. Cooke and P. A. Buckley, Eds. (Academic Press, London, 1987), pp. 163–198], we cloned restriction fragments of manakin DNA into bacteriophage λ or plasmid vectors and used randomly selected recombinant clones as DNA hybridization probes in Southern blotting to screen for RFLP markers. The λ5–Bgl II probe-enzyme combination detects a 5.6-kb band in *M. vitellinus* that is cleaved into a 3.7- and a 1.9-kb band in *M. candei*; the pSCN3–Ava II combination detects a 5.5-kb band in *M. candei* that is cleaved into a 4.8- and a 0.7-kb band in *M. vitellinus* (both combinations reveal additional bands that are not diagnostic). Heterozygotes were indicated by the presence of all three marker bands.
14. One-way analysis of variance (ANOVA) on sample data from localities 1 through 7 indicates no significant variation for beard length and tail length ($P > 0.5$) [Minitab Data Analysis Software, release 6.1.1, Minitab Inc., 1987]. Fisher's exact test on allele frequencies from localities 1 through 7 indicates no significant variation for pSCN3–Ava II ($P = 0.94$) or λ 5–Bgl II ($P = 0.07$) (SAS Procedures Guide, release 6.07, SAS Institute, Carey, NC, 1991).
15. Any spatially varying pattern within a population could in principle be due to a complex, spatially varying pattern of selection forces or environmental determinants. Given the genetic evidence of hybridization and the lack of any apparent habitat heterogeneity, our data are most simply explained by introgression of golden-collared traits as a result of hybridization.
16. A. Lill, *Z. Tierpsychol.* **36**, 1 (1974).
17. If female *M. candei* prefer golden collars, traits not originally present in males of their species, sexual selection may be based on preexisting sensory or cognitive biases, rather than on coevolution between female preference and the preferred trait [M. J. Ryan, in *Oxford Surveys in Evolutionary Biology*, D. Futuyma and J. Antonovics, Eds. (Oxford Univ. Press, New York, 1990), vol. 7, pp. 157–195; M. J. Ryan and A. S. Rand, *Evolution* **44**, 305 (1990); A. L. Basolo, *Science* **250**, 808 (1990)]. Intrasexual selection could also be based on such preexisting biases.
18. Beards are male secondary sexual characteristics in *Manacus*, yet the longer beard of *M. vitellinus* has not introgressed together with the plumage color traits. This suggests that selection for golden collars can predominate over any selection that may be occurring on beard length, and that the gene (or genes) for beard length are not closely linked to those for plumage color. Alternatively, the lack of introgression may be due to tight linkage of beard length to loci with alleles that are negatively selected in hybrids.
19. That introgression of golden collars stops at the Río Changuinola may be a coincidence or may indicate that the river creates a dispersal barrier over which golden-collared traits have not crossed. Genetic differentiation across wide rivers in South America has been documented in other manakins [A. P. Capparella, *Acta XIX Congressus Internationalis Ornithologici* **2**, 1658 (1988)].
20. Collar width was measured directly. Yellowness of underparts was ranked on a scale from 1 to 5, with reference specimens selected to define the integer points of the scale; a score of 5.0 was defined by an *M. candei* specimen with uniform yellow underparts, 1.0 by an *M. vitellinus* specimen with gray-green underparts (reference series United States National Museum (USNM) accession numbers: 5.0, 608192; 4.0, 608168; 3.0, 608186; 2.0, 606944; 1.0, 13636). Throat color was ranked similarly; the white throats of *M. candei* were assigned a score of 6.0, 5.0 was defined by a locality 3 bird with a lemon-yellow throat, and 1.0 by a locality 10 bird with a golden-orange throat (reference series USNM accession numbers: 6.0, 608192; 5.0, 608158; 4.0, 608167; 3.0, 606915; 2.0, 608146; 1.0, 608136).
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Evidence of DNA Bending in Transcription Complexes Imaged by Scanning Force Microscopy

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Complexes of *Escherichia coli* RNA polymerase with DNA containing the λ 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 bent in open promoter complexes containing RNA polymerase 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 λ 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 use 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 $MgCl_2$ 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 λ 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 sitting astride the template with the flanking arms of the DNA meeting underneath the polymerase.

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