Genetic Restoration of the Florida Panther

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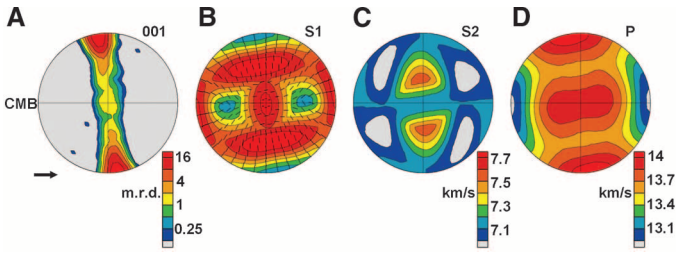

Fig. 3. Equal area projection pole figures showing texture development and anisotropic elastic properties of a pPv aggregate in a subducting slab near the core-mantle boundary using deformation mechanisms established in this study. **(A)** (001) pole figure of pPv showing a snapshot during spreading in a plane strain regime (i.e., intermediate between pure shear and simple shear). **(B)** Fast shear-wave velocities and polarization. **(C)** Slow shear-wave velocities. **(D)** *P* wave velocity surface. Flow direction indicated by arrow.

deformation. Deformation of pPv has been extensively modeled using the viscoplastic self-consistent polycrystal plasticity code [VPSC; (*31*)] (*16*, *17*, *23*). A comparison of the IPFs obtained in this study with results of VPSC models shows that the 001 texture observed here is compatible with dominant slip on (001) lattice planes and 40% compressive strain (Fig. 2D). For this model, the transformation texture obtained after conversion to pPv was used as the starting texture for the deformation simulation (Fig. 2A).

Admittedly, there are limitations to our experiments because the time scale of deformation, grain size, composition, temperature, and deviatoric stress are quite different from those expected in the *D″*. However, if we assume that slip is active on (001) planes in $MgSiO_3$ pPv at *D″* conditions and combine this information with geodynamic modeling of deformation along the *D″*, we can predict texture development in the lowermost mantle. This can, in turn, be combined with single crystal elastic constants to assess associated seismic anisotropy. For these calculations, we neglect any contribution from the second most abundant mineral in the mantle, ferropericlase, which may also play an important role in generating *D″* anisotropy (*32*).

In this context, we use information from the same two-dimensional geodynamic model applied previously (*17*, *33*) to predict texture development in a slab subducted into the *D″* zone. A tracer records the strain-temperature history and, accordingly, the texture evolution is modeled with the same polycrystal plasticity theory applied to the experiment (*31*). It is assumed that the aggregate has a random orientation distribution as it enters the *D″* about 290 km above the CMB. Based on our results, we assume dominant (001)[100] and (001)[010] slip. We chose a geodynamic tracer that records strain and temperature, which advances close to the CMB and attains large strains. Preferred orientation develops rapidly and then stabilizes; note the strong alignment of (001) lattice planes slightly inclined to the CMB (Fig. 3A). By averaging the orientation distribution and single crystal elastic properties (*34*), we calculated aggregate elastic properties and seismic wave propagation. Based on the polarization directions of the fast and slow shear-wave velocities, high shear-wave splitting is 0.55 km/s in the flow direction; fast shear waves are polarized parallel to the CMB (Fig. 3B, C). This is consistent with seismic observations of the circum-Pacific regions (*2*, *4*), where the presence of pPv is expected (*35*, *36*). The anticorrelation between fast *S* waves and *P* waves in the flow direction (Fig. 3D) is also consistent with seismic records (*37*, *38*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5999/1639/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 and S2
References

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Genetic Restoration of the Florida Panther

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The rediscovery of remnant Florida panthers (*Puma concolor coryi*) in southern Florida swamplands prompted a program to protect and stabilize the population. In 1995, conservation managers translocated eight female pumas (*P. c. stanleyana*) from Texas to increase depleted genetic diversity, improve population numbers, and reverse indications of inbreeding depression. We have assessed the demographic, population-genetic, and biomedical consequences of this restoration experiment and show that panther numbers increased threefold, genetic heterozygosity doubled, survival and fitness measures improved, and inbreeding correlates declined significantly. Although these results are encouraging, continued habitat loss, persistent inbreeding, infectious agents, and possible habitat saturation pose new dilemmas. This intensive management program illustrates the challenges of maintaining populations of large predators worldwide.

Pumas (also called cougars, mountain lions, or panthers) are currently distributed throughout western North America and much of Central and South America (*1*). The endangered Florida panther (listed in 1967, table S1), the last surviving puma subspecies in eastern North Amer-

ica, is restricted to shrinking habitat between the urban centers of Miami and Naples (Fig. 1). By the early 1990s, the population of ~20 to 25 adults (*2*) showed reduced levels of molecular genetic variation relative to other puma populations (*3*–*5*), which is indicative of inbreeding (*4*, *6*). This may have led to defects, including poor sperm quality and low testosterone levels (*4*, *7*), poor fecundity and recruitment (*4*, *7*), cryptorchidism [where >80% of males born from 1990 to 1992 had one or no descended testes (*4*)], a high incidence of thoracic cowlicks and kinked tails (*4*), numerous atrial septal defects (*4*, *8*), and a high load of parasites and infectious disease pathogens (*4*, *8*–*10*).

In 1995, these cumulative observations, coupled with demographic models predicting a 95% likelihood of extinction within two decades, motivated the translocation of 8 wild-caught Texas (TX) female pumas into habitat occupied by at least 22 adult canonical (last-remaining, authentic) Florida panthers (CFPs) and 4 Everglades Florida panthers (EVGs) (Fig. 1A), because historically, gene flow occurred between Texas and Florida puma populations (*11*, *12*).

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We compared data from 591 individuals sampled from 1978 to 2009 (table S2). Twenty-three informative (minimum allele frequency > 0.1) short tandem repeat (STR) loci were examined to reconstruct genetic heritage and parentage relationships; assess spatial and demographic patterns; distinguish CFP from other puma lineages; track morphological, biomedical, and life history traits as indices of fitness; and associate genetic heritage and heterozygosity with panther survival (*13*).

Pumas of diverse ancestry, time periods, and geographic origins, including wild-caught and captive animals in Florida, clustered into phylogenetic groups (Fig. 2). This analysis, combined with Bayesian population genetic results from STR genotypes that revealed nine distinct groups (fig. S1 and table S2), allowed us to explicitly infer the genetic heritage of each Florida panther (table S2, column 2; Figs. 1 and 2; and fig. S2) and to distinguish the two pre-1995 groups (CFP and EVG). Further, admixed Florida panthers (AdmFPs) were clearly identifiable, including first-generation (F_1) offspring of TX females bred by CFP or EVG males (CFPxTX-F_1 or EVGxTX-F_1) and panthers that were related to captive pumas of western U.S. origin who had escaped from enclosures on the Big Cypress Seminole Indian Reservation (SEM) from 1997 to 1999 (Figs. 1 and 2).

From 1986 to 1995, the minimum number of adult (>1.5 years old) panthers fluctuated from 24 to 32 (Fig. 3A), and genetic heritage remained relatively stable (85% CFP and 15% EVG; Fig. 1 and figs. S1 and S3). After their introduction, five of eight TX females bred (Fig. 1a) and produced 15 F_1 kittens with CFP, EVG, and at least five TX-backcross (TX-BC) offspring (figs. S3 and S4 and table S2). Twelve F_1 panthers produced offspring. From 1995 to 2008, 424 panther births were documented (81 CFP, 319 AdmFP, and 24 undetermined; 272 were observed only as neonatal kittens). These largely AdmFP were responsible for colonizing former panther range and densities increased. For example, between 1995 and 2007, the number and density of panthers in the southern Big Cypress National Preserve (BCNP) (2174 km^2) increased eightfold from 3 (0.14/100 km^2) to 25 (1.15/100 km^2) (Fig. 1).

From 1996 to 2003, numbers (N) increased by 14%/year to at least 95 adults (Fig. 3A), 26.6 kittens were produced annually (fig. S2). The effective population size (N_e) rose from 16.4 in 1995 to 32.1 by 2007, and N_e/N was 0.314 (Fig. 1) (*13*). This paralleled an increase in average individual STR heterozygosity (to 25% from 18.4% in 1993; Fig. 3B) and a decrease in the average estimated age of adults from 6.6 to 4.2 years from 1997 to 2004. Population growth slowed and average age increased gradually after 2004 (Fig. 3C).

Admixed genetic ancestry was associated with increased survival of F_1, EVG-BC, and TX-BC kittens (<1 year old) relative to purebred CFP and CFP-backcross (CFP-BC) kittens (0.518 ± 0.130 versus 0.243 ± 0.074, $P = 0.020$) (Fig. 3D). F_1 adults had significantly higher survival ($P = 0.002$) than other admixed or CFP groups (table S4), with a risk ratio (RR: relative instantaneous probability of mortality) of 0.118 (*13*). The survival of subadults and adults increased significantly with heterozygosity (RR for an increase of 0.1 = 0.643, $P = 0.011$). Interestingly, CFPs also experienced significantly higher mortality rates from intraspecific aggression than did AdmFPs (RR = 3.077, $P = 0.014$), and mortality rates from intraspecific aggression declined as heterozygosity increased (RR = 0.480, $P = 0.005$) (*13*).

Demographic differences among >1-year-old panthers from 2002 to 2004 (Fig. 3A) were evident when 23 out of 29 (23/29) (79%) CFPs alive in 2002 were lost versus 22/47 (47%) AdmFPs and when CFPs had a significantly

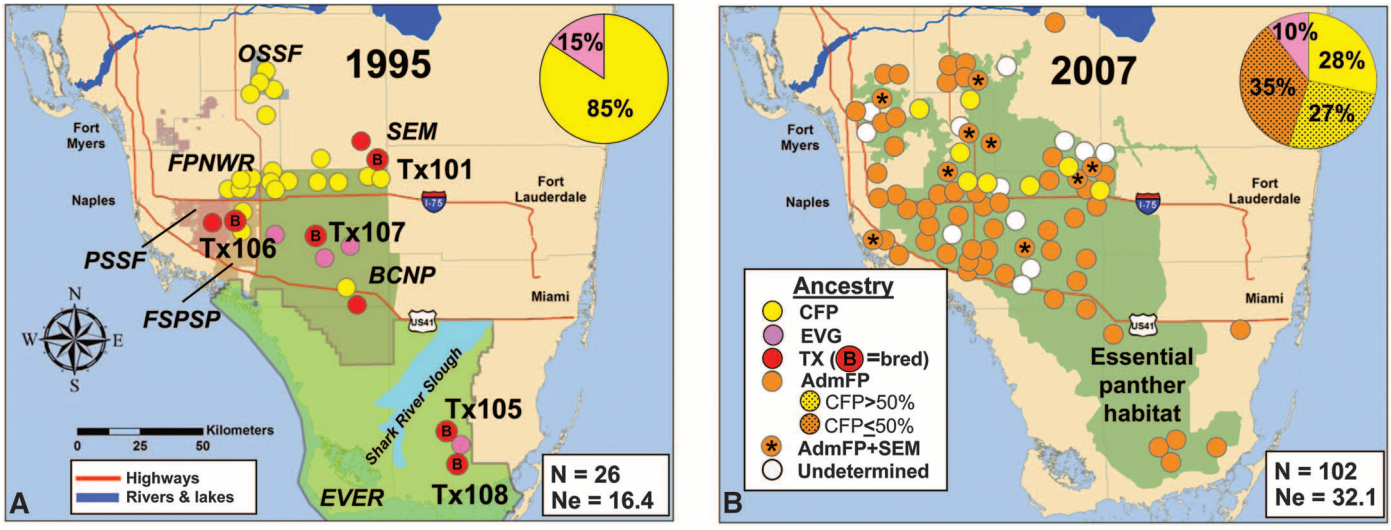

Fig. 1. (**A** and **B**) Southern Florida (1995, left; 2007, right) with locations of breeding-age Florida panthers (>1.5 years old), geographic features, number (N) alive, and effective population size (N_e). Labeled colored areas in (A) demarcate public land (*23*): Fakahatchee Strand Preserve State Park (FSPSP), Picayune Strand State Forest (PSSF), Florida Panther National Wildlife Refuge (FPNWR), BCNP, Big Cypress Seminole Indian Reservation (SEM), Okaloacoochee Slough State Forest (OSSF), and Everglades National Park (EVER) and in (B) show panther habitat. Circles are coded by ancestry: CFP, TX females (with a B if a successful breeder), EVG, AdmFP, and SEM. Pie charts illustrate the genetic heritage of the population (fig. S1 and table S2) (*13*).

lower yearly survival (likelihood-ratio test χ^2 = 5.38, P = 0.020), dropping from 0.827 ± 0.044 (from 1997 to 2001) to 0.610 ± 0.087 [AdmFP survival declined from 0.904 ± 0.046 to 0.866 ± 0.027 (P = not significant)]. The number of documented CFP kittens went from 17 in 2002 to 5 total from 2003 to 2005, and none have been observed since (fig. S2). The CFP contribution to the AdmFP population also decreased, with only four litters (14 kittens) of CFPxAdmFP parents documented after 2004. The abrupt CFP decline (versus AdmFP increases; Fig. 3A) and differential patterns of survival and mortality are consistent with an AdmFP competitive advantage.

Panther survival is also affected by disease agents (*4*, *9*). From 2001 to 2007, 19 Florida panthers tested positive for feline leukemia virus (FeLV) antibodies, and 5 in Okaloacoochee Slough State Forest (OSSF) (Fig. 1) died with active FeLV infections (*10*). A capture and vaccination program was implemented in 2003, and no further active infections were documented after July 2004. Further, the prevalence of a puma-specific strain of feline immunodeficiency virus (FIV_{PCO}) increased 16 to 80% from 1995 to 2005. Although not explicitly implicated in clinical disease in free-ranging pumas, FIV_{PCO} infection may predispose individuals to other diseases due to low lymphocyte numbers (*14*).

A detailed population pedigree confirmed all dam/offspring inferences from field observations (278 kittens from 128 litters marked as neonates, and 51 juveniles and subadults associated with suspected dams), supported 120 of 130 sire/offspring field inferences, and identified an additional 174 probable parents (48 dams and 126 sires). At least one parent was assigned to 422 individuals: 74 different dams and 49 sires to 397 and 298 offspring, respectively (table S2 and figs. S3 and S4). The estimated relative genetic contribution of the TX females to the descendant population varied widely (TX101 = 0.20, TX105 = 0.01, TX106 = 0.06, TX107 = 0.10, and TX108 = 0.04).

Shrinking and fragmented populations are at high risk for inbreeding depression (*15*, *16*) and local extinction (*17*) through demographic and stochastic events (*18*). These influences probably caused the precipitous decline in Florida panther N_e from 1900 to 1980 (*19*). The stated goal of the Florida panther genetic restoration plan was to improve population size and viability by increasing genetic variability without losing unique local adaptations. By several measures, this experiment was successful. Most notably, after the introduction of Texas females, the population tripled, with a parallel significant reduction in the incidence of several phenotypic characters historically associated with inbreeding depression (Table 1). Additionally, admixed panthers exhibited behaviors that might be associated with higher fitness, as evidenced by increased escape behavior from high trees during capture (RR = 2.0, P = 0.001, fig. S5).

In addition to genetic restoration, enhancement in panther numbers was probably facilitated by action initiated in the late 1980s by federal, state, and private groups to mitigate panther declines and facilitate natural recovery. This included buttressing legal protection under the Endangered Species Act (*20*), acquiring and protecting >120,000 ha of occupied panther habitat, altering prey management (*21*), and constructing highway underpasses to reduce mortality from vehicle strikes (*22*). In

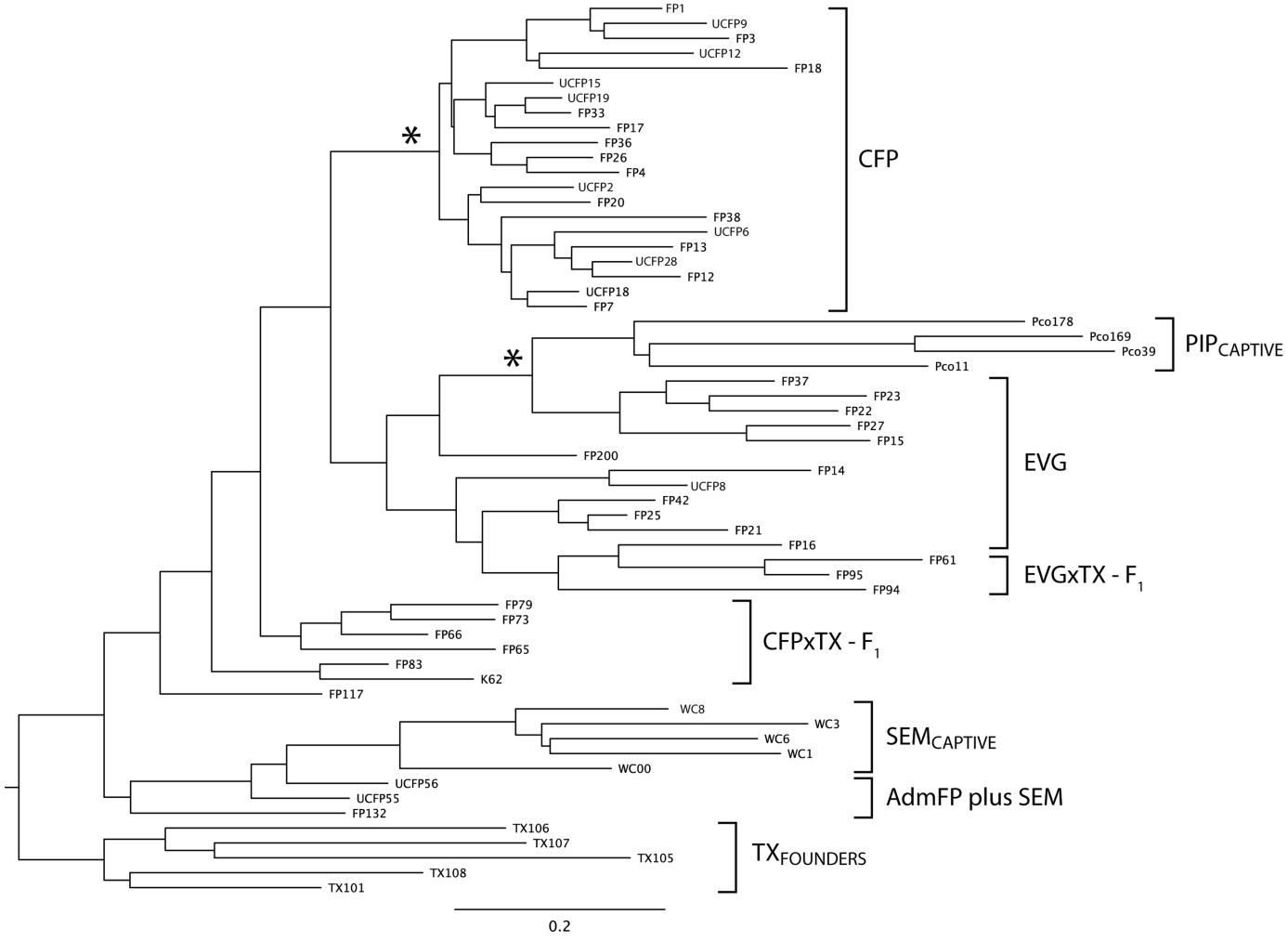

Fig. 2. Neighbor-joining tree of composite STR genotypes portraying genetic relationships among pre-1995 founding CFPs, introduced TX females, EVG and F_1 admixed Florida panthers (CFPxTX-F_1 and EVGxTX-F_1). Captive Piper panthers ($PIP_{captive}$) are related to present-day EVGs through individuals released into the Everglades between 1957 and 1967 (*3*, *4*), and SEMs contributed unintended gene flow. Asterisks mark nodes with >80% bootstrap support (*13*).

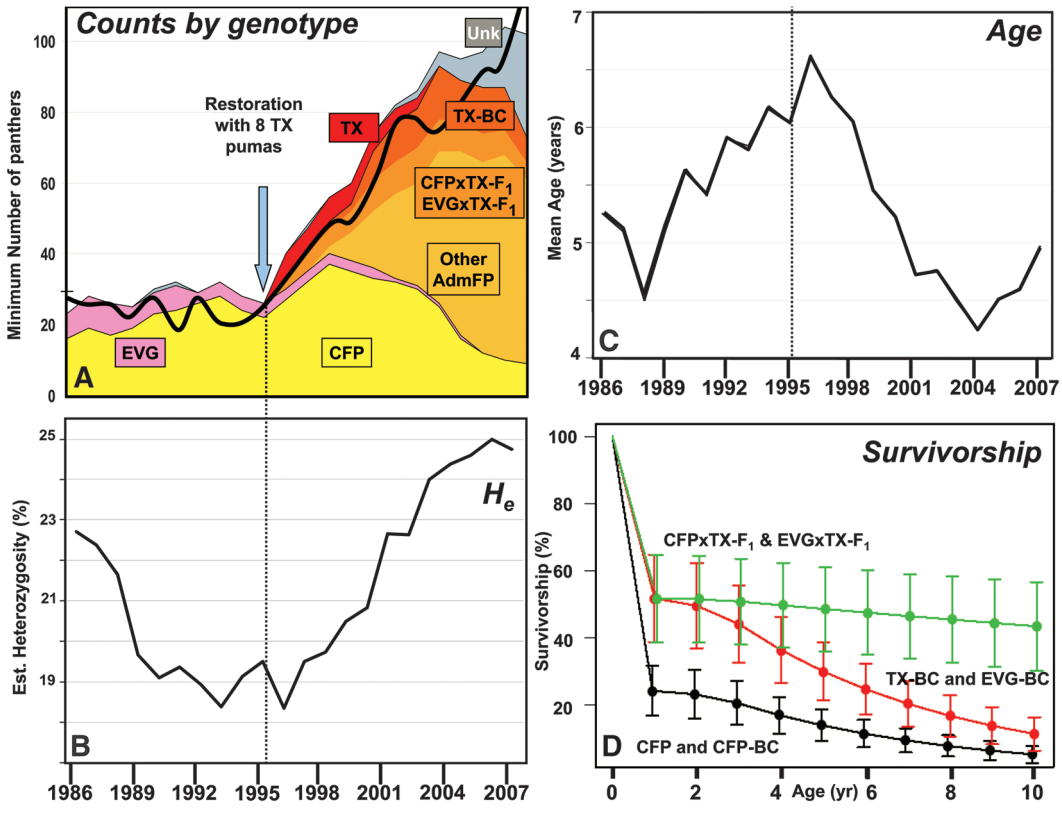

Fig. 3. (**A**) Minimum annual subadult and adult panther population size and inferred genetic heritage from 1986 to 2007. CFPs are yellow, EVGs pink, TXs red, CFPxTX-F_1s and EVGxTX-F_1s orange, TX-BCs and other AdmFPs shades of orange, and genetically uncharacterized individuals (Unk) gray (*13*). The black line is an independent minimum-count estimate from surveys of tracks, spoor, and other field evidence (*2*). (**B**) Mean yearly adult multilocus heterozygosity. (**C**) Yearly mean age of adults. (**D**) Projected survivorship (probability of surviving to an age) curves for female Florida panthers of different genetic heritages with standard error bars (*13*). Male trends are similar (table S4).

Table 1. Estimates of molecular genetic variation and prevalence of physiological and morphologic traits in Florida panthers of different genetic heritages (*13*) (see table S3). NA, not available.

			Crytorchidism**			Prevalence of**			
Heritage group	No.	Average* heterozygosity	Avg. no. of descended testicles	Prevalence in males	Normal sperm (%) Ejaculate (EJ) Gamete rescue (GR) (No. of males)	Atrial septal defects	Kinked tails	Cowlick on thorax	Percent of** individuals with ≥1 abnormal trait
CFP	116	0.167 ± 0.005^{A}	1.3 ± 0.07^{A}	0.66 ± 0.06^{A}	EJ 5.4 ± 0.7^{C} (15) GR 10.1 ± 1.9^{N} (13)	0.17 ± 0.05^{A}	0.90 ± 0.03^{A}	0.81 ± 0.04^{A}	70.3 ± 2.5^{A}
EVG	17	0.282 ± 0.022^{B,D}	2.0 ± 0^{B}	0^{B}	9.5 ± 0.6^{B} (5)	0^{A}	0.31 ± 0.12^{B}	0.29 ± 0.11^{B}	22.5 ± 7.1^{B}
TX	5	0.318 ± 0.02^{B,D}	2.0 ± 0^{B}§	0^{B}§	14.0 ± 3.5^{A,B}§ (9)	0^{A}	0^{B}	0^{B}	0^{B}
All AdmFP	143	0.244 ± 0.006^{B}	1.9 ± 0.0^{B}	0.10 ± 0.035^{B}	(See below)	0.08 ± 0.030^{A}	0.25 ± 0.037^{B}	0.27 ± 0.039^{B}	19.5 ± 2.1^{B}
AdmFP groups									
TX-F_1	10	0.336 ± 0.01^{B,}	2.0 ± 0	0^{B}	EJ 20.5 ± 4.5^{A} (2)	0^{A}	0^{B}	0.38 ± 0.18^{B}	14.6 ± 7.3^{B}
TX-BC	18	0.273 ± 0.016^{B,D}	2.0 ± 0^{B}	0^{B}	NA	0.14 ± 0.14^{A}	0^{B}	0.06 ± 0.06^{B}	3.2 ± 2.2^{B}
Other AdmFP	52	0.251 ± 0.008^{C,D}	1.94 ± 0.04^{B}	0.06 ± 0.042^{B}	EJ 7.0 ± 6.0 (2) GR 16.6 ± 3.2^{P} (10)	0.065 ± 0.045^{A}	0.160 ± 0.05^{B}	0.22 ± 0.0^{B}	14.4 ± 3.2^{B}
Non–TX-BC	63	0.216 ± 0.008^{C}	1.8 ± 0.06^{B}	0.17 ± 0.06^{B}	NA	0.08 ± 0.04^{A}	0.43 ± 0.070^{B}	0.36 ± 0.07^{B}	29.4 ± 3.3^{B}

*t test and **Fisher's exact test: Column values (mean ± SE) with different superscript letters (A to D; except GR sperm, which is N and P) are significantly different ($P < 0.05$). §From (*7*).

spite of improvements, ongoing density-dependent factors (related to limited and decreasing habitat availability) and stochastic events will continue to regulate population growth, requiring continued commitments to identify and maintain additional quality habitat to preserve Florida panther evolutionary potential for the long term.

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Supporting Online Material

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Parasympathetic Innervation Maintains Epithelial Progenitor Cells During Salivary Organogenesis

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The maintenance of a progenitor cell population as a reservoir of undifferentiated cells is required for organ development and regeneration. However, the mechanisms by which epithelial progenitor cells are maintained during organogenesis are poorly understood. We report that removal of the parasympathetic ganglion in mouse explant organ culture decreased the number and morphogenesis of keratin 5–positive epithelial progenitor cells. These effects were rescued with an acetylcholine analog. We demonstrate that acetylcholine signaling, via the muscarinic M1 receptor and epidermal growth factor receptor, increased epithelial morphogenesis and proliferation of the keratin 5–positive progenitor cells. Parasympathetic innervation maintained the epithelial progenitor cell population in an undifferentiated state, which was required for organogenesis. This mechanism for epithelial progenitor cell maintenance may be targeted for organ repair or regeneration.

Organogenesis involves the coordinated growth of epithelium, mesenchyme, nerves, and blood vessels, which use common sets of genes, guidance cues, and growth factor–signaling pathways (*1*–*5*). Research on epithelial organogenesis has focused on epithelial-mesenchymal and endothelial-epithelial cell interactions. However, the function of the peripheral nervous system during epithelial organogenesis is less clear.

Pavlov's seminal experiments on dogs demonstrated that neuronal input controls salivary gland function (*6*), and more recent work showed that parasympathetic innervation of salivary glands is essential for regeneration after injury (*7*). Because parasympathetic innervation occurs in parallel with salivary gland development (*8*), we hypothesized that parasympathetic innervation is required for epithelial progenitor cell function during organogenesis.

To test this hypothesis, we used mouse embryonic submandibular gland (SMG) explant culture and mechanically removed the parasympathetic submandibular ganglion (PSG) before the gland developed (*9*). SMG development begins at embryonic day 11 (E11), when the oral epithelium invaginates into neural crest–derived mesenchyme (*10*). The neuronal bodies of the PSG condense around the epithelium at E12 (fig. S1A) and could be separated from epithelium and mesenchyme in explant culture. When the separated tissues were recombined in culture, the growth of the SMG epithelium was reduced, with a significant decrease in the number of end buds in the absence of the PSG (Fig. 1, A and B, and fig. S2A). The PSG axons have abundant varicosities (fig. S2B, box) that contain the neurotransmitter acetylcholine (ACh) (*8*), and express the ACh synthetic enzyme (*Chat*) (fig. S1, B and C). ACh activates epithelial muscarinic (M) receptors, and M1 (*Chrm1*) is the major muscarinic receptor in the embryonic SMG epithelium (fig. S1, B and C), whereas M1 and M3 (*Chrm3*) stimulate saliva secretion in the adult (*7*). Alternatively, we perturbed ACh/M1 signaling using the chemical inhibitors, 4-DAMP (DAMP; *N*-2-chloroethyl-4-piperidinyl diphenylacetate), an irreversible M1/M3 inhibitor (Fig. 1C); atropine, a competitive muscarinic antagonist (fig. S2D); beta-bungarotoxin (Btx), which depletes neuronal ACh stores (fig. S2E); and small interfering RNA (siRNA) to M1 (*Chrm1*) (fig. S2, H to I). All treatments reduced the number of end buds (fig. S2, C to I). In contrast, inhibition of α_2-adrenergic receptors with idazoxan had no effect (fig. S2F). These experiments demonstrate that epithelial morphogenesis requires PSG, ACh, and M1 activity.

Epithelial morphogenesis may also depend on the size of the epithelial progenitor pool and growth factor–mediated proliferation (*11*). To distinguish between these two possibilities, we mea-

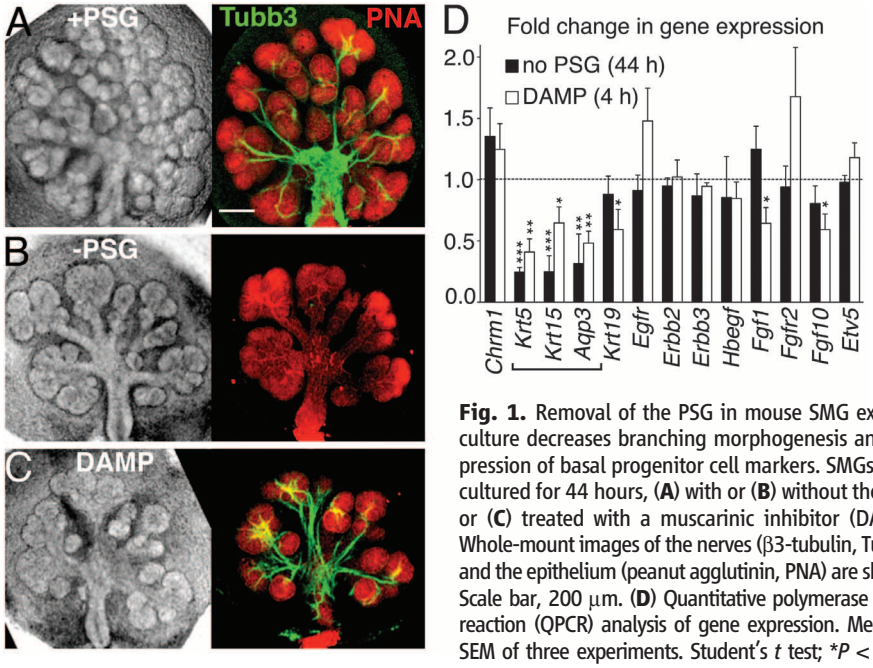

Fig. 1. Removal of the PSG in mouse SMG explant culture decreases branching morphogenesis and expression of basal progenitor cell markers. SMGs were cultured for 44 hours, (**A**) with or (**B**) without the PSG or (**C**) treated with a muscarinic inhibitor (DAMP). Whole-mount images of the nerves (β3-tubulin, Tubb3) and the epithelium (peanut agglutinin, PNA) are shown. Scale bar, 200 μm. (**D**) Quantitative polymerase chain reaction (QPCR) analysis of gene expression. Means ± SEM of three experiments. Student's *t* test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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Researchers have had more success in developing hypotheses about Compositae's evolutionary sequence (phylogeny) by using molecular techniques to study genetic similarities among existing species. These studies initially identified two related families: Goodeniaceae, centered in Australia; and Calyceraceae, which are in southern South America and are closer to Compositae (*6*, *7*). Calyceraceae, a small family of just four genera and about 60 species (*8*), stands in contrast to the huge sunflower assemblage of 1500 genera. The phylogenetic approach has helped researchers identify what appears to be the oldest known familial relative: Barnadesiinae, a little known subtribe of Mutisieae; it consists of nine genera and 91 species from southern and Andean South America (*9*, *10*).

All data suggested that the common ancestor of Goodeniaceae, Calyceraceae, and Compositae developed in Antarctica, when it had mixed temperate and tropical forests (*11*, *12*). As Antarctica cooled during Eocene-Oligocene (~56 million to 23 million years ago) (*13*), the ancestral form dispersed and migrated eastward into Australia, resulting eventually in Goodeniaceae, and westward into southern South America, leading to the progenitor of Calyceraceae and Compositae. The splitting of Calyceraceae and Compositae, therefore, would have occurred in southern South America during the Eocene (~56 million to 34 million years ago). These hypotheses have been buttressed by "molecular clock" studies, which suggest that Compositae diverged in the Eocene, approximately 50 million years ago (*14*).

Given this background, Barreda *et al*.'s report of an Eocene fossil from southern South America showing clear flowering heads with phyllaries and pappus is important. At long last, there is clear macrofossil evidence of the sunflower family at an early stage of its diversification, just where it had been hypothesized to originate. The fossil does not allow unequivocal assignment, but the authors suggest that its large, conical heads and types of pappus and phyllaries are broadly compatible with Mutisieae. There is also dispersed pollen found in the matrix with the fossil, and its features are also suggestive of Mutisieae (or possibly Carduoideae). Detailed scanning and transmission electron microscopic studies on the pollen would be helpful for deeper understanding of relationships, as would finding pollen in situ in anther sacs of a better preserved fossil.

Much remains to be learned about the evolution and biogeography of the sunflower family. A new book (*15*) has synthesized molecular phylogenetic studies and, in consort with the new fossil reported here, provides strong stimuli for further research. Even if researchers accept the sunflower's origin in southern South America, it is still unclear how the family quickly colonized the entire planet and became so incredibly diverse.

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GENETICS

A Bit of Texas in Florida

Florida's inbred panthers benefited from the import of Texas pumas.

Craig Packer

Harassed, hunted, and restricted to ever smaller areas, most populations of large carnivores are fragmented into archipelagoes of parks and reserves. Biologists have long warned of the negative genetic consequences of inbreeding in such small populations. To restore genetic health, they have prescribed "active management," including moving, or translocating, individuals into inbred populations. In a time of budget cuts and inadequate funding for effective conservation, however, is translocation worth the costs? Moving a lion from Namibia to South Africa is not a trivial exercise, nor is the translocation of cougars from one part of the United States to another. But it may be worth the trouble, Johnson *et al*. (*1*) report on page 1641 of this issue. In the most comprehensive study ever conducted on the effects of inbreeding in wild carnivores, they find convincing evidence that the "quality" of a population of Florida panthers was successfully improved by the addition of panthers from Texas.

Florida panthers (also called cougars, pumas, or mountain lions) have been studied in considerable detail since the 1970s and provide an exceptionally clear example of the genetic consequences of prolonged inbreeding. By the early 1990s, Florida's population of 20 to 25 adult panthers was showing lower genetic variation than other puma populations. Biologists observed a range of problems—including heart defects, poor sperm quality, poor fecundity, and many adult males with one or no descended testes—that led to predictions that the population could go extinct within decades. In a bid to stem the tide, managers introduced eight female Texas cougars to Florida in 1995.

By comparing genetic data collected from 591 Florida panthers between 1978 and 2009, Johnson *et al*. show that Texas-Florida hybrid offspring have replaced the original inbred stock. The researchers documented increased levels of genetic heterozygosity (having different versions of the same gene), and the hybrid offspring enjoyed greater viability and fewer genetic abnormalities. The adult hybrids were also superior competitors: The pure-bred Florida panthers suffered greater mortality from fights with outbred cougars, and hybrids were better able to climb trees when pursued by scientists.

The size of the panther population has also increased since the translocation, but this result is more difficult to interpret. The amount of land available to the Florida panther has increased in the past few decades due

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Gene flow. The Florida panther got a genetic boost from introductions of pumas from Texas, but other big cats still face serious problems around the world.

to a variety of conservation measures. More land supports more cougars, so it is hard to estimate how much of the population growth resulted from the influx of "fresh blood" as opposed to range expansion. A similar translocation of 16 lions into a highly inbred population in Hluhluwe iMfolozi Park, a fenced reserve in South Africa, also improved the reproductive performance of the lions in the park, but population size did not increase in the short term (*2*).

Although translocation looks to be an effective technique for ameliorating the genetic consequences of small population size, the larger problem still remains. Big cats may be popular in places where they've become scarce and most people live in cities, but the rest of the world still struggles to deal with the dangers that man-eaters and cattle-killers pose to rural residents. Lions attacked more than 100 Tanzanians every year for the first few years of this millennium (*3*), and thousands of livestock are killed by lions, leopards, and jaguars throughout the world each year.

Retaliatory poisoning is increasingly common in Africa, greatly reducing the number of large carnivores outside national parks, and trophy hunting is excessive and poorly regulated, resulting in rapid population declines in many jurisdictions (*4*). Even in the United States, attitudes toward cougars vary from state to state. Montana paid bounties for dead cougars between 1908 and 1911; the take averaged about 140 animals per year. In contrast, between 1997 and 1999, trophy hunters in Montana killed an average of 800 cougars per year—virtually at the same time as the translocation from Texas to Florida. In 2006, Oregon announced plans to increase trophy hunting in order to decrease the state's cougar population by 40% and thereby reduce livestock depredation.

Although 21st-century Floridians may be willing to enlarge panther habitat, the story is still quite different in the rest of the world. We can perhaps take some consolation from Johnson *et al.*'s study: Once the entire planet reaches the same state of economic development and urbanization as the United States, wildlife managers all over the world can look forward to carting rare species from one park to another until the end of time.

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GENETICS

Exposing a DUX Tale

A DNA sequence stabilizes the expression of a gene that may affect muscle development and lead to muscular dystrophy.

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Facioscapulohumeral muscular dystrophy (FSHD), the third most common muscular dystrophy, is characterized by progressive weakness that starts in the facial muscles, proceeds to the upper back (scapula) and shoulder-upper arm regions (humeral), and eventually affects the trunk and lower extremities. Since 1992, this disorder has been associated with an array of repeated DNA sequences (called D4Z4) on chromosome 4 (*1*). An unaffected chromosome 4 has between 11 and more than 100 repeat units within D4Z4, but when this is shortened to 1 to 10 units, disease develops (see the figure). How this contraction leads to disease has been a mystery. Over the past 3 years, analyses of chromosome 4q35 have identified a combination of DNA sequences (haplotype 4A161) associated with susceptibility to FSHD, suggesting that specific sequence variations are coupled to disease pathogenesis in conjunction with D4Z4 contraction (*2*). On page 1650 of this issue, Lemmers *et al.* (*3*) provide an intriguing unifying model for FSHD pathogenesis based on very high resolution haplotype mapping and sequence analyses and careful study of exceptional pedigrees.

FSHD pathogenesis has been one of the most puzzling enigmas in human genetics for the past two decades, but there was always a consensus that the disease was caused by a gain-of-function mutation (*1*). Each D4Z4 repeat unit has a sequence called *DUX4* that potentially encodes a double homeobox gene putatively involved in developmental regu-

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