

stains⁷. Thus, the amount of cortex devoted to each appendage can be accurately determined.

To quantify the sensory input from each appendage, one half of the nose from each of four moles was thin-sectioned at a level where a single nerve branch was visible for each appendage (*b* in the figure). Each nerve branch was photographed and the myelinated fibres in each were counted. The cortex from the corresponding hemisphere of each mole was flattened, sectioned tangential to the surface at 60–90 μm , and stained with cytochrome oxidase to reveal the 11 bands representing each half nose (*c*).

The sizes of the cortical representations of the nasal appendages are not proportional to their peripheral innervation densities (*d*). The 11th appendage, which is preferentially used to explore objects, is greatly over-represented in cortex, occupying more than 25% of the nose field. In addition, appendages 1, 9 and 10, which are also used during feeding⁸, show disproportionate cortical expansions. The 11th appendage is allocated approximately 130 μm^2 of cortex per afferent, whereas most of the other appendages are allocated between 30 and 50 μm^2 per afferent. Similar results have been obtained for the visual system in rhesus monkeys, where ganglion cells in the retinal fovea occupied 3.3–5.9 times more cortical area than ganglion cells in the peripheral retina⁴.

These results are the first report of a disproportionately expanded cortical rep-

resentation of peripheral inputs in the somatosensory system and they raise important questions about the development and maintenance of cortical areas. Although the results are consistent with the findings in the somatosensory cortex of primates, where additional magnification of cortical areas occurs in response to increased peripheral stimulation⁹, the expanded areas in the mole somatosensory cortex are not the result of an experimental manipulation. Because of the long evolutionary history of this functional fovea, we could ask whether there are phylogenetic contributions to this cortical specialization, or whether the preferential cortical magnification is simply the result of the regional differences in patterns of stimulation across the sensory surfaces during ontogeny (and thereafter). This question might be addressed in neonatal star-nosed moles.

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Sex of the last wild Spix's macaw

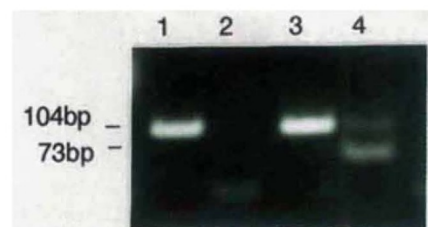
SIR—The Spix's macaw (*Cyanopsitta spixii*) is the world's most endangered bird¹. Only 32 now survive and since 1987 only one individual has remained in the wild. Coupled with a programme of releases, it is hoped that this individual will help found a new wild population. Unfortunately, the Spix's macaw, like most bird species, is sexually monomorphic. Although behavioural observations suggest that the wild bird is male, this has never been verified. It is essential to have this information if a prospective mate is to be released.

Confirmation of the sex of the wild Spix's macaw would normally involve its capture, but this course has been ruled out as a significant risk to the bird. The only apparent alternative would be to use a test based on the polymerase chain reaction (PCR)² to identify the sex of this individual from the DNA traces present in moulted feathers³. Unfortunately, no such technique has been described applicable to this, or indeed any other, bird species.

Birds exhibit female heterogamety: that is, females have one W and one Z sex

chromosome whereas males have two Z chromosomes. Thus, the presence of a W-unique genetic marker is diagnostic of the female sex. We recently described a technique to isolate sex-specific genetic markers⁴ and have used this method to identify a highly conserved gene (*C-W*) from the chicken which seems to be W-linked in all birds, with the possible exception of the primitive ratites (our unpublished data). A second closely related gene (*C-2*) is situated elsewhere in the genome.

Stratagene provided a genomic library from the closely related hyacinth macaw (*Anodorhynchus hyacinthinus*), and from this we isolated a homologue of *C-W* and designed PCR primers to amplify a 104-base-pair (bp) region of both *C-W* and *C-2* from Spix's macaw DNA. Sequence determination revealed that the *C-W*-derived PCR product possessed a *DdeI* restriction enzyme site which was absent in the *C-2* product. Thus, PCR amplification and *DdeI* cleavage of male Spix's macaw DNA yields only a single product of 104 bp, whereas from female DNA two products are apparent, one of 104 and one of 73 bp. The presence of the *C-2* product in both



DdeI-restricted PCR products showing that the remaining wild Spix's macaw is male. Lane 1, wild bird; 2, negative extraction control; 3, known male; 4, known female. The larger fragment is 104 bp long and the female W-chromosome-specific fragment is 73 bp.

METHODS. DNA from the wild bird was extracted⁵ from 1-cm portions of the tips of 3 moulted flight feathers collected over the past 2 years. The negative extraction control was taken through an identical procedure. 1.5% of these extraction products or 50 ng genomic DNA from the reference samples were subjected to semi-nested PCR. Primary amplification consisted of 20 cycles with primers (5' to 3') P3, AGATATTCGGATCTGATAGTGA, and P2, TCTGCATCGCTAAATCCTTT; 1% of the primary PCR product was subjected to 30 cycles of amplification with P2 and P1, ATATTCTGATCTGATAGTGA(C/T)TC. Samples were denatured for 1.5 min at 95 °C, then cycled between 57 °C per 30 s, 72 °C per 15 s and 94 °C per 30 s with a 5-min final extension. Products were precipitated, cut with *DdeI*, reprecipitated and electrophoresed through a Visigel separation matrix (Stratagene). All appropriate precautions and negative controls⁵ were implemented. The accuracy of the test was confirmed using DNA from Spix's and hyacinth macaws of known sex ($n = 5$, $P = 0.03$). Uncut secondary PCR product from the wild bird was isolated⁶, cloned using the Stratagene pCR-Script SK(+) kit and sequenced (Amersham: 7-deaza-dGTP kit) to confirm that the product had originated from a Spix's macaw. We thank Stratagene, M. Kelsey, P. W. H. Holland and J. R. Krebs for advice and assistance. *C-W* and *C-2* are subject to patent application 9413821.1.

sexes acts as a control to ensure the PCR amplification has been successful.

We extracted DNA from feathers moulted by the wild Spix's macaw using a technique devised to purify ancient DNA⁵. The PCR-based test described above was used to demonstrate that *C-W* was not present in the sample (see figure). This confirmed that the wild bird is male. A female Spix's macaw has recently been released as a prospective mate.

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