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Conservation biology

Restoration of an inbred adder population

The negative effects of inbreeding on population size are well documented in captive animals[1], but there is surprisingly little evidence that genetic factors cause a decline in wild populations[2,3], apart from a reported correlation of low levels of genetic variability with a high incidence of malformed or stillborn offspring[4]. From the point of view of conservation strategies, it is not only the effect of genetic factors on population decline that needs to be considered, but also whether introducing novel genes can prevent or reverse such a decline. Here we show that the introduction of new genes into a severely inbred and isolated population of adders (*Vipera berus*) halted its precipitous decline towards extinction and expanded the population dramatically.

We studied an isolated population of adders at Smygehuk, about 50 km south of Lund in Sweden. The area is bordered to the south by the Baltic Sea, to the north by arable land, to the west by a village and to the east by a harbour. The adders are confined to a coastal strip of grassy meadow 1 km long and 50–200 m wide. They have been isolated from other adder populations for at least a century, and the nearest known population is 20 km to the north across agricultural fields unsuitable for adders[4].

The population declined dramatically around 35 years ago[4] and has since suffered from severe inbreeding depression, with a high proportion of deformed or stillborn offspring and very low genetic variability[4]. Since 1981, all adders captured in the study area have been marked by clipping of the ventral scales. The small size and open habitat of the study area means that all adult males can be captured during spring (late March to early May) each year, when they bask in the open, enabling us to count the new recruits (recently matured males).

Our estimates of population number are based on adult males only, as these can be most reliably censused. Female adders in our study area have an approximately biennial reproductive cycle. Non-reproductive females emerge from hibernation up to four weeks later than reproductive females and exhibit cryptic behaviour, making them much harder to find in spring than males and reproductive females. In any given year, therefore, we are unable to find all non-reproductive females, so counting male snakes provides the most robust indicator of population dynamics.

The population size at Smygehuk has fallen each year since 1983 (the decline from 1981 to 1995 is highly significant: $r = -0.83$, $P = 0.0001$, d.f. = 14; Fig. 1a), mainly because there are fewer recruits (correlation between the total number of males captured each year and the number of recruiting (unmarked) males: $r = 0.79$, $P = 0.0001$, d.f. = 17). The population was lowest in 1995, when only four males were caught.

In spring 1992, we captured 20 adult male adders from large and genetically variable populations north of Smygehuk and released them into the Smygehuk population. They remained there for four mating seasons, and the eight surviving snakes were captured and released back into their natal populations in 1995. The introduced males are not included in our data on population demography (Fig. 1a).

The introduced males settled in rapidly, and mated with all the reproductive female adders in Smygehuk from their first season[4]. Because male adders mature at about four years of age[5], we did not expect to see any effect on the number of adult males until 1996, which is what occurred. From 1996 to 1999, there were dramatic increases in the number of recently matured males recruiting to the population ($r = 0.96$, $P = 0.04$, d.f. = 3) and the total number of males captured ($r = 0.98$, $P = 0.02$, d.f. = 3), which peaked at 32 in 1999, the most recorded over the 19-year study (Fig. 1a).

The genetic variability within the population also increased rapidly from 1996 to 1999. We isolated DNA from whole blood and analysed polymorphism in the MHC class I by restriction-fragment length polymorphism[6]. Before the new males were introduced, the population had extremely low genetic variability (Fig. 1b). The recently recruited males exhibited much more genetic variability (Fig. 1b), confirming that most of them were sired by the introduced males. The proportion of stillborn offspring also fell suddenly[4], indicating that the rapid increase in recruitment was due to increased survival of juvenile adders.

The isolated nature of the population precludes immigration, so the only plausible alternative would require an increase in the number of litters produced. However, the number of females reproducing each year was slightly lower during the recovery phase than during the earlier decline (mean, 8.2 litters per year from 1981 to 1991 compared with 4.8 litters per year from 1992 to 1995).

Our population data from 1983 to 1995 indicate that the Smygehuk adders were on the brink of extinction, with falling numbers and negligible recruitment (Fig. 1a). Introducing new genes from a different population enabled the adders to make a dramatic recovery. This result encourages genetic approaches to conservation and

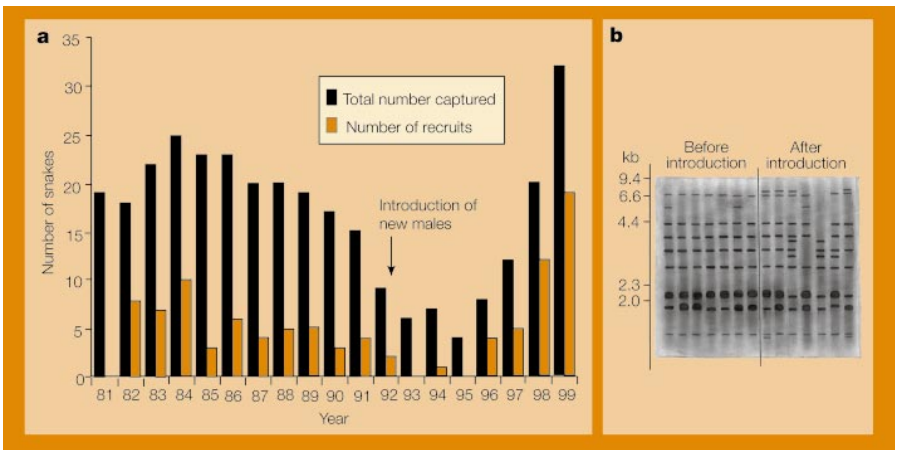

Figure 1 Introducing new males increases the genetic diversity and enables the adder population to recover. **a,** Total number and number of recruited male adders captured in Smygehuk from 1981 to 1999. **b,** Southern-blot analysis of major histocompatability complex (MHC) class I genes in seven males sampled before the introduction of new males (left) and in seven recruited males sampled in 1999 (right).

supports the importance of preserving genetic variability as a way of increasing the viability of wild populations.

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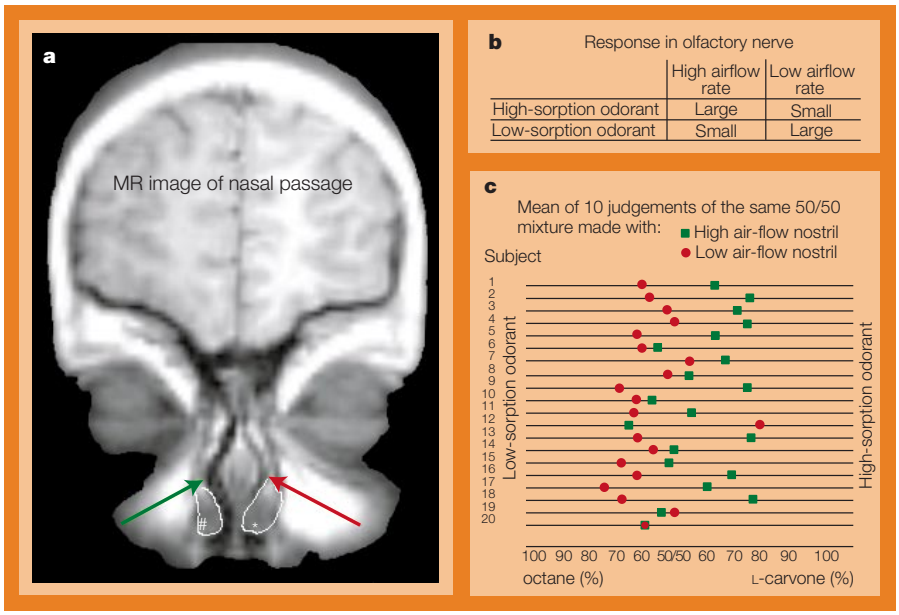

Figure 1 Different nostrils convey different olfactory information to the brain. **a,** Magnetic resonance image of the nasal passage, which appears dark. The swollen (*) and relaxed (#) turbinates, outlined in white, result in an occluded right nostril (red arrow) and a clearer left nostril (green arrow). **b,** The interaction between airflow rate and odorant sorption, which brings about a response in the olfactory nerve[10]. **c,** On each of ten trials, subjects were asked to smell an identical mixture of 50% octane and 50% L-carvone using either the left or right nostril. They were then given each individual odorant component to smell separately and judged the composition of the mixture by marking the line (experimental sequences were randomized and counterbalanced). Using the high-flow-rate nostril (green), the average judgement was that the mixture consisted of 55% L-carvone and 45% octane. Using the low-flow-rate nostril (red), the judgement was that it consisted of 61% octane and 39% L-carvone ($t(19) = 3.74$, $P = 0.001$). For the 20 subjects, there was no significant group difference in airflow rate between the left and right nostrils, but there was a significant group difference between the high-flow-rate nostril and the low-flow-rate nostril (high mean = 51 l min^{-1}, low mean = 31 l min^{-1}, $t(19) = 5.6$, $P < 0.0001$).

Olfaction

The world smells different to each nostril

The flow of air is greater into one nostril than into the other because there is a slight turbinate swelling in one[1–3]. The nostril that takes in more air switches from the left to the right one and back again every few hours[4–6], but the effect of this switching on the sense of smell has been unclear[7,8]. Here we show that this difference in airflow between the nostrils causes each nostril to be optimally sensitized to different odorants, so that each nostril conveys a slightly different olfactory image to the brain.

The slight swelling that obstructs each nostril (Fig. 1a) causes odorants to be drawn into the nostrils at different rates. But for an odorant to act on the olfactory receptors, it must first cross the olfactory mucosa. Different odorants sorb to and cross the mucosa at different rates[9]. In the bullfrog, for example, a specific odorant's sorption rate interacts with the rate of airflow across the mucosa to produce varying amplitudes of response in the olfactory nerve[10]. A high-sorption odorant induces a smaller response when airflow is low and a larger one when it increases. In contrast, a low-sorption odorant induces a smaller response at a high airflow rate and a larger response when there is less airflow (Fig. 1b).

This occurs because, when a high-sorption odorant has a low airflow rate, the odorant molecules sorb to the mucosa before moving very far along it. Only a small portion of the epithelium is involved in the response, which is small. When the same odorant flows at a high airflow rate, it spreads across a larger mucosal area before sorbing, so the response is larger. When a low-sorption odorant flows quickly, it moves past the mucosa without sorbing so the epithelial response is small. When the same low-sorption-odorant flows slowly, it has time to sorb across the mucosa and the response is larger[10].

We therefore investigated whether the nostril with the higher airflow in humans is the more sensitive to high-sorption odorants and the nostril with lower airflow more sensitive to low-sorption odorants. We used an olfactometer to produce an equally proportioned mixture of the high-sorption odorant L-carvone and the low-sorption odorant octane. The mixture was always the same but subjects were told that it was slightly different for every trial.

Subjects sampled the mixture by sniffing with one nostril (the other nostril was occluded) and made a judgement about the relative proportion of the two components in the mixture (for example, 55% octane and 45% L-carvone). The task was repeated for the second nostril and the judgements compared. The rate of airflow for each sniff was measured by anterior rhinomenometry. We found that 17 of 20 subjects (binomial, $P = 0.001$) thought the mixture contained more octane when they used the low-airflow nostril, and more L-carvone when they used the high-airflow nostril (Fig. 1c).

The nostril with the higher airflow reverses periodically[4–6], so we tested eight subjects after the nostril with greater airflow had switched, and found that the perception of the same mixture reversed in seven of the eight subjects (binomial, $P = 0.035$). Odorant perception was therefore dependent on airflow rate, not on whether the odorant was smelled by the left or right nostril.

The different airflow between the nostrils results in a disparity of olfactory perception. Providing the olfactory system with two disparate images of the olfactory world with each sniff in this way may improve olfactory acuity by expanding the range of odorants that are within optimal sensitivity in a given sniff.

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