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**Authors' contributions** D.G. conducted analyses and discovered the original pattern, and drafted the manuscript together with B.C.S., who also provided overall guidance, and L.E.B.K., who also advised over quantitative genetic analyses. T.A.W. conducted spatial analyses. R.H.McC. maintained the long-term database.

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## Gene flow maintains a large genetic difference in clutch size at a small spatial scale

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Understanding the capacity of natural populations to adapt to their local environment is a central topic in evolutionary biology. Phenotypic differences between populations may have a genetic basis, but showing that they reflect different adaptive optima requires the quantification of both gene flow and selection<sup>1–3</sup>. Good empirical data are rare<sup>4</sup>. Using data on a spatially structured island population of great tits (*Parus major*), we show here that a persistent difference in mean clutch size between two subpopulations only a few kilometres apart has a major genetic component. We also show that immigrants from outside the island carry genes for large clutches. But gene flow into one subpopulation is low, as a result of a low immigration rate together with strong selection against immigrant genes. This has allowed for adaptation to the island environment and the maintenance of small clutches. In the other area, however, higher gene flow prevents local adaptation and maintains larger clutches. We show that the observed small-scale genetic difference in clutch size is not due to divergent selection on the island, but to different levels of gene flow from outside the island. Our findings illustrate the large effect of immigration on the evolution of local adaptations and on genetic population structure.

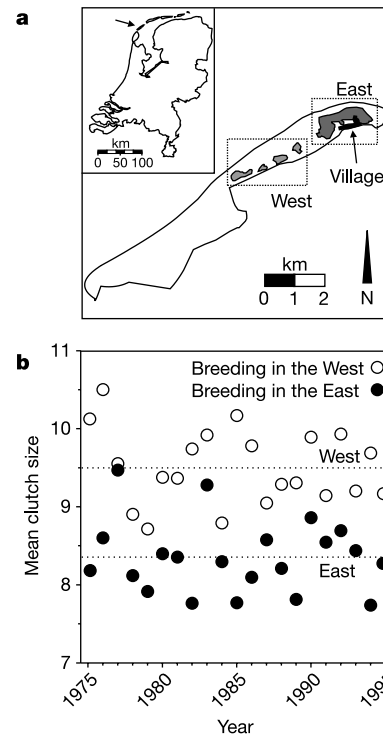
Gene flow plays a crucial role in the evolution of natural populations<sup>2,3</sup>. Although its role may be beneficial (by counteracting the negative effects of genetic drift and inbreeding on genetic variation<sup>5,6</sup>), its role in constraining evolution by homogenizing the gene pool gains most attention<sup>1,7</sup>. In spite of a wealth of theoretical studies showing that gene flow counteracts genetic differentiation, and thus the evolution of local adaptations, good empirical evidence is scarce, particularly for quantitative traits in spatially structured populations<sup>4</sup>. Those empirical studies that addressed the impact of gene flow on the evolution of local adaptations have focused on only some aspects, and investigated,

for example, the performance of populations inhabiting two different environments<sup>8,9</sup>, or the effect of immigration into a single population<sup>10–12</sup>. Furthermore, in the majority of studies assumptions had to be made regarding the genetic component in the phenotypic variation observed, or levels of selection and gene flow were not quantified (but see ref. 13).

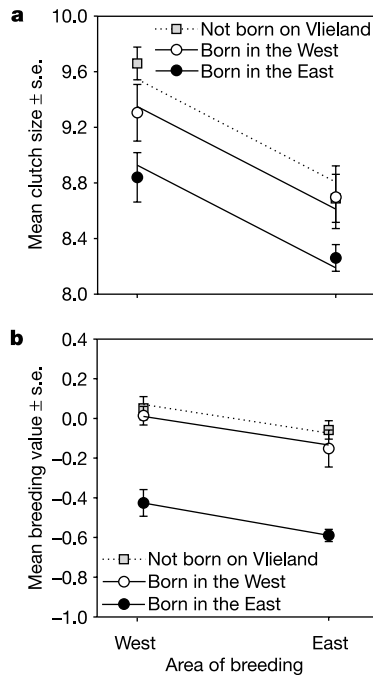
A fascinating case of phenotypic variation at a small spatial scale can be found on the island of Vlieland in the Netherlands (Fig. 1a), where over the period 1975–95 females that bred in the western part of the island laid  $1.15 \pm 0.14$  eggs more than females that bred in the eastern part (paired *t*-test:  $t_{20} = 8.55$ ,  $P < 0.001$ ), which is 0.91 s.d. of the average within-area and within-year distribution (Fig. 1b). Here we analyse what proportion of this persistent difference can be attributed to genetic variation, and then investigate the roles of immigration and several components of selection in maintaining this difference.

The classic approach to test for local adaptations, and genetic differences in general, is a common garden or other transplant experiment. Although such experiments are relatively easy to perform in a laboratory setting<sup>14,15</sup>, or in sessile organisms in the wild<sup>16</sup>, they are not feasible for the majority of wild animals (but see refs 13, 17). However, about 10% of the females born on Vlieland breeds in the other area (also, see below), which allows for a separation of genetic and environmental effects. Additionally, the properties of this long-term study population allow for an accurate estimation of both immigration and selection (see Methods).

Whereas the difference in clutch size between the western and eastern part of the island ('East' and 'West', respectively) can partly be attributed to the area of breeding, and thus to phenotypic plasticity, approximately half of the difference is accounted for by where a female is born, which suggests a genetic component to the difference in clutch size between the West and the East (Fig. 2a). Although females that have dispersed to the other side of the island may either represent a non-random subset of birds or breed, for



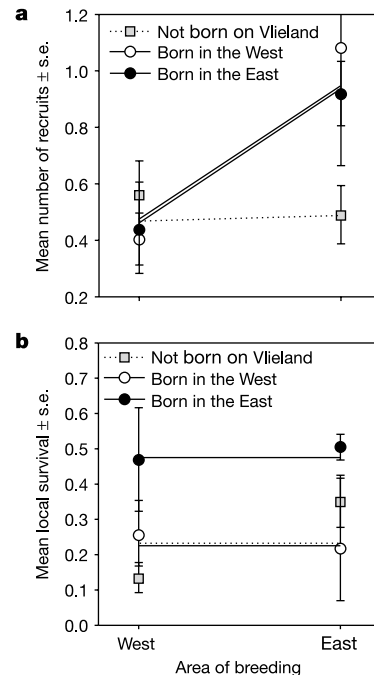
**Figure 1** Clutch sizes on the island of Vlieland. **a**, Map of Vlieland, with the location of the woodlands that provide suitable nesting habitat in grey, and their grouping into West and East. **b**, Yearly mean clutch size in the West and the East from 1975 to 1995. Dotted lines give the overall mean.



**Figure 2** Spatial variation in clutch size. **a**, Mean clutch sizes of females in relation to origin and area of breeding (born in the West or the East: origin:  $F_{1,67} = 6.43$ ,  $P = 0.014$ ; area of breeding:  $F_{1,67} = 11.0$ ,  $P = 0.002$ , interaction:  $F_{1,66} = 0.01$ ,  $P = 0.94$ ). Immigrants do not differ significantly from females born in the West: ( $t_{108} = 1.15$ ,  $P = 0.25$ ), but they do from females born in the East (after Tukey–Kramer adjustment for multiple comparisons:  $t_{108} = 3.86$ ,  $P = 0.006$ ). **b**, Mean predicted breeding values for clutch size for males in the same categories (born in the West or the East: origin:  $F_{1,56} = 57.9$ ,  $P < 0.001$ ; area of breeding:  $F_{1,56} = 8.11$ ,  $P = 0.006$ ). Immigrants do not differ significantly from males born in the West: ( $t_{82} = 1.03$ ,  $P = 0.31$ ), but they do from males born in the East (after Tukey–Kramer adjustment for multiple comparisons:  $t_{82} = 10.02$ ,  $P < 0.001$ ).

example, in poorer territories, this should affect birds from the West and the East similarly, and result in crossing instead of parallel reaction norms. Moreover, we find a very similar difference in the mean predicted breeding values for clutch size of males (see Methods) born in the West and the East (Fig. 2b), which argues against an environmental effect of the area of birth lasting throughout an individual's life. On the whole, we have strong evidence for a genetic difference in clutch size of approximately 0.5 egg between birds born in the West and the East.

To understand how this large genetic difference could be maintained over a period of 21 yr at such a small spatial scale, and more specifically whether this difference reflects different adaptive optima, we first compared the viability and fecundity of birds born in the West and the East breeding in either of the two areas. This provides us with the most direct and general test for local adaptation to the environment in the West and the East. Although both males and females produce about twice as many recruits (see Methods) when they breed in the East than in the West (males: year:  $F_{20,36} = 2.59$ ,  $P = 0.006$ ; area of breeding:  $F_{1,36} = 20.86$ ,  $P < 0.001$ ; females: year:  $F_{20,47} = 4.18$ ,  $P < 0.001$ ; area of breeding:  $F_{1,47} = 11.9$ ,  $P = 0.001$ ), there is no significant difference between birds born in the West and the East (males:  $F_{1,35} = 0.005$ ,  $P = 0.95$  (Fig. 3a); females:  $F_{1,46} = 2.79$ ,  $P = 0.10$ ). Local survival (see Methods) of females born in the East is higher than that of females born in the West, irrespective of where they breed (Fig. 3b). The difference in local survival between males born in the West and the East is not significant (year:  $F_{1,57} = 1.04$ ,  $P = 0.44$ ; area of breeding:  $F_{1,57} = 8.18$ ,  $P = 0.021$ ; area of birth:  $F_{1,57} = 1.38$ ,  $P = 0.18$ ), and is significantly different from that in females (model including both

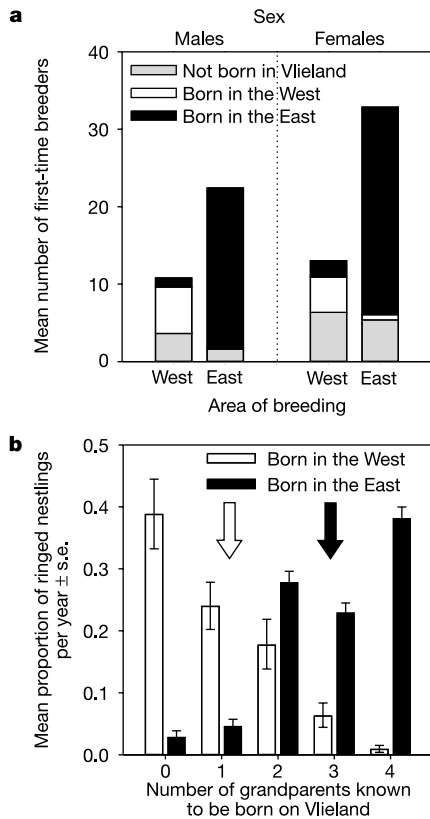


**Figure 3** Variation in fitness in relation to origin and area of breeding. **a**, Mean number of recruits produced in one year for local and immigrant males, and breeding in the West or the East (year:  $F_{20,54} = 3.25$ ,  $P < 0.001$ ; local or immigrant  $\times$  area of breeding:  $F_{1,54} = 11.3$ ,  $P = 0.001$ ; Local versus immigrant in the West:  $t_{54} = 1.37$ ,  $P = 0.18$ , and in the East:  $t_{54} = 3.32$ ,  $P = 0.002$ ). **b**, Mean local survival of females (born in the West or the East: year:  $F_{20,48} = 1.88$ ,  $P = 0.037$ ; origin:  $F_{1,48} = 6.61$ ,  $P = 0.013$ ; area of breeding:  $F_{1,47} = 0.04$ ,  $P = 0.85$ ). Immigrants do not differ significantly from females born in the West: ( $t_{89} = 0.078$ ,  $P = 0.94$ ), but they do from females born in the East (after Tukey–Kramer adjustment for multiple comparisons:  $t_{89} = 2.86$ ,  $P = 0.015$ ).

sexes: sex  $\times$  area of birth:  $F_{1,103} = 8.22$ ,  $P = 0.005$ ). So, local survival of females born in the East is twice as high as that of females born in the West, both in the West and the East. This indicates that females born in the East are better adapted to the environment on Vlieland in general than females born in the West, and argues against local adaptation of birds to their area of birth.

To investigate the role of immigration from outside Vlieland, we compared their clutch size and fitness to birds born in the West and the East, and quantified the number of immigrants into both parts of the island. The inclusion of immigrants into the analyses above shows that immigrant females lay significantly larger clutches than females born in the East (Fig. 2a), and that immigrant males have significantly higher predicted breeding values for clutch size than males born in the East (Fig. 2b). Furthermore, immigrant females have a significantly lower local survival than females born in the East (Fig. 3b). In neither of these cases, however, can they be distinguished from birds born in the West. Finally, when immigrant males breed in the East they produce significantly fewer recruits than males born in both the East ( $t_{68} = 3.03$ ,  $P = 0.0035$ ) and the West ( $t_{68} = 2.15$ ,  $P = 0.035$ ) (Fig. 3a). In females this difference in recruitment between immigrants and locals in the East is absent (born on Vlieland or not  $\times$  area of breeding:  $F_{1,60} = 0.05$ ,  $P = 0.82$ ), and significantly different from the difference between local and immigrant males (model including both sexes: sex  $\times$  born on Vlieland  $\times$  area of breeding:  $F_{1,134} = 4.76$ ,  $P = 0.031$ ).

The fact that birds born in the West are genetically very similar to immigrants, at least with respect to clutch size, can be understood from the fact that the proportion of first-year breeders that is not born on Vlieland is on average 3.3 times higher in the West (43%) than in the East (13%) (Fig. 4a). This, together with the relatively

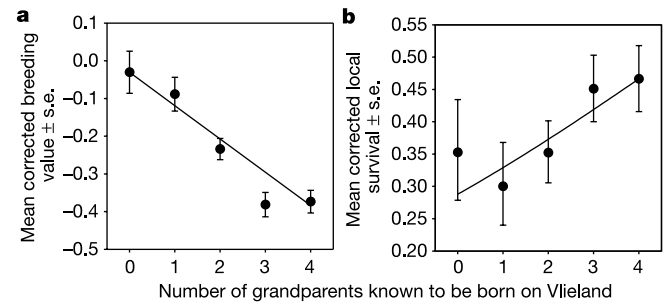


**Figure 4** Immigration into the East and the West. **a**, The average composition of the breeding population for both the West (mean proportion of immigrants among first-time breeders = 43%, 95% CI: 35–51%) and the East (mean = 13%, 95% CI: 10–16%). **b**, The proportion of all ringed nestlings per year against the number of local grandparents for both the West (mean across all young ringed as nestlings =  $1.07 \pm 0.098$ ; open arrow) and the East (mean =  $2.85 \pm 0.049$ ; filled arrow).

higher recruitment of immigrants in the West, results in a much higher level of gene flow into this part of the island, and as a consequence birds that are born in the West are more closely related to immigrants than birds born in the East (Fig. 4b).

On the basis of these findings, we propose that the following mechanism underlies the genetic difference between birds born in the West and the East: immigrants carry the genes for relatively large clutches. Both in the East and the West there is selection against immigrant genes in females, and in the East there is additional selection against immigrant males. In the Eastern sub-population this, together with the lower immigration rate, is maintaining local adaptation to the environment on Vlieland, as well as smaller clutches. The higher level of gene flow in the West results in larger clutches, and prevents local adaptation. This mechanism is supported by two findings, which are directly predicted from Figs 2b and 3b, respectively: within the males born on Vlieland, those males that are less related to immigrants have genes for smaller clutches (Fig. 5a). Furthermore, of the females born on Vlieland, those females that are less related to immigrants have a higher local survival (Fig. 5b).

We have shown that great tits not born on Vlieland have genes for larger clutches, and have a lower survival on Vlieland. However, within the East there is no evidence for viability selection against large clutches when we correct for a female's origin (year:  $\chi^2_{(20)} = 61.8$ ,  $P < 0.001$ ; area of birth:  $\chi^2_{(2)} = 6.09$ ,  $P = 0.048$ ; standardized clutch size:  $\chi^2_{(1)} < 0.005$ ,  $P = 0.96$ ,  $|\beta'| < 0.001$ ) (see Methods), which argues against a causal relationship between the two. The lower survival of immigrant genotypes is thus not directly related to clutch size. Furthermore, across the period



**Figure 5** Clutch size and fitness in relation to an individual's relatedness to immigrants. **a**, The relationship between predicted breeding values for clutch size of males and the number of local grandparents ( $b = -0.088 \pm 0.015$ ,  $F_{1,535} = 36.5$ ,  $P < 0.001$ ), after correction for an effect of origin ( $F_{1,535} = 23.8$ ,  $P < 0.001$ ) and the area of breeding ( $F_{1,535} = 5.42$ ,  $P < 0.001$ ). **b**, The relationship for females between local survival and the number of local grandparents ( $\chi^2_{(1)} = 4.98$ ,  $P = 0.027$ ), corrected for an effect of year ( $\chi^2_{(20)} = 53.69$ ,  $P < 0.001$ ) and area of birth ( $\chi^2_{(1)} = 8.10$ ,  $P = 0.004$ ).

1975–95 there is no relationship between clutch size and the number of recruits produced (year:  $\chi^2_{(20)} = 126.2$ ,  $P < 0.001$ ; standardized clutch size:  $\chi^2_{(1)} = 0.63$ ,  $P = 0.43$ ,  $|\beta'| < 0.001$ ). Across the years before 1975, however, there is a clear trend that females that laid small clutches produced more recruits (year:  $\chi^2_{(15)} = 61.5$ ,  $P < 0.001$ ; standardized clutch size:  $\chi^2_{(1)} = 3.46$ ,  $P = 0.063$ ,  $\beta' = -0.14$ ). This relatively strong selection<sup>18</sup> could explain how the small clutches of birds born in the East have evolved in the first place, but also the potential for founder effects should not be ruled out.

Great tits on Vlieland maintain their small clutches against 13%, but not against 43%, immigration. Although phenotypic variation across environments is often assumed to be adaptive<sup>19</sup>, we show that a scenario involving different levels of gene flow can maintain genetic differences in a life-history trait for a highly mobile animal at a very small spatial scale (see also ref. 20). Our results illustrate the major consequences of immigration for genetic population structure and fitness. Even though Vlieland is relatively isolated when compared to many mainland populations<sup>21</sup>, within-island movements have a negligible effect. The properties of the Vlieland population have allowed us to disentangle the role of genetic and environmental variation, and of gene flow and selection. There is, however, no reason to believe that similar processes would not take place in many more populations where they would remain undetected. □

## Methods

### Data set

The data were collected from the nest-box breeding population of great tits on the island of Vlieland (53.17° N, 5.03° E), The Netherlands. The first nest boxes were put up in 1955, and the population has been monitored continuously since then. On the basis of exchange rates among the five separate woodlands, the population can be divided into an eastern and a western sub-population<sup>22,23</sup>. Both subpopulations are at least 1.3 km, and on average 5.1 km, apart (Fig. 1a). Unless specified otherwise, analyses are performed on first clutches of first year breeders from 1975 until and including 1995. Clutch size is assumed to be a trait of the laying female (see Supplementary Information). Manipulated clutches were included in the analyses (see Supplementary Information). There was no temporal trend in mean clutch size over this period, either in the West (linear regression:  $b = -0.013 \pm 0.018$ ,  $t_{19} = -0.72$ ,  $P = 0.48$ ), or in the East ( $b = -0.010 \pm 0.017$ ,  $t_{19} = -0.56$ ,  $P = 0.58$ ) (Fig. 1b).

Nest boxes are provided in excess in all suitable nesting habitat. Females breeding in either the East or the West that had not been ringed as a nestling anywhere on Vlieland are considered to be immigrants, although a small proportion of those may have been born on Vlieland<sup>21</sup>. This will, however, make the majority of our tests more conservative. The degree of relatedness of birds born on Vlieland to immigrants is expressed in how many of their four grandparents had been ringed as nestlings on Vlieland. Immigrants are assumed to have parents who were not born on Vlieland. Birds for which the origin of any of their parents or grandparents could not be inferred were excluded. The mean number of grandparents born on Vlieland of birds born in the East or the West did not change significantly over time (linear regression:  $b = 0.0047 \pm 0.010$ ,  $t_{19} = 0.46$ ,  $P = 0.65$ ).

## Statistical analyses

To account for the non-independence of individual observations within a year, and the large differences in sample size among groups, analyses were, where possible, performed on yearly means for the relevant sub-group. When appropriate, yearly means were square-root or arc-sine transformed before analysis<sup>24</sup>. All presented means and parameter estimates are back-transformed.

All statistical analyses were performed using the SAS statistical package using the GLM and GENMOD procedures<sup>25</sup>. Non-significant interactions were removed first, starting with the least significant, followed by non-significant single terms, again starting with the least significant. When interactions were significant, the main effects were kept in the model, regardless of their significance. All tests are two-tailed.

## Selection analyses

Local survival was used as a measure of viability, which is defined as the probability that a bird is observed breeding again on Vlieland the next year. Local recruitment was used as a measure of fecundity, and is defined as the number of offspring produced in all clutches in a year observed to be breeding on Vlieland in subsequent years. We can therefore not distinguish between mortality and emigration of both fledglings and adults. Both processes do however have a similar effect on the population level. Only clutches from which at least one chick fledged were included in analyses of recruitment.

To quantify selection acting on clutch size, we calculated standardized selection gradients ( $\beta'$ ) by regressing relative fitness (fitness divided by the mean fitness in that year) on standardized clutch size (clutch size relative to the mean in that year, divided by the standard deviation)<sup>26</sup>. Significance of selection gradients was determined from a generalized linear model with binomial and Poisson errors, using standardized clutch sizes. If significant, origin of birth was included in the model. Selection gradients were calculated for both the period from 1955 until 1975, and for 1975 until 1995. As a result of the small sample sizes in the West, especially in the first period, analyses were limited to females breeding in the East.

## Animal model analysis

Genetic parameters were estimated using an animal model, which uses all available information on both ancestors and descendants to separate an individual's phenotype into an additive genetic component (or breeding value) and other random and fixed effects<sup>27,28</sup>. The amounts of variance accounted for by the random effects (additive genetic variance  $V_A$ , permanent environmental variance  $V_{PE}$ , and residual variance  $V_R$ ) were estimated using a Restricted Maximum Likelihood (REML) technique implemented in the software package VCE4<sup>29</sup>, and were equal to 0.58, 0.42 and 0.94, respectively. The narrow sense heritability  $\pm$  s.e. of clutch size on Vlieland across the period 1965–2003 (defined as  $V_A/V_P$ ) was  $0.30 \pm 0.028$ . Best Linear Unbiased Predictions (BLUPs) of breeding values were obtained for all individuals in the pedigree using the software package PEST<sup>30</sup>. See Supplementary Information for more details on pedigree reconstruction and which fixed and random effects were included in the animal model.

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## A mechanism for impaired fear recognition after amygdala damage

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Ten years ago, we reported that SM, a patient with rare bilateral amygdala damage, showed an intriguing impairment in her ability to recognize fear from facial expressions<sup>1</sup>. Since then, the importance of the amygdala in processing information about facial emotions has been borne out by a number of lesion<sup>2–4</sup> and functional imaging studies<sup>5,6</sup>. Yet the mechanism by which amygdala damage compromises fear recognition has not been identified. Returning to patient SM, we now show that her impairment stems from an inability to make normal use of information from the eye region of faces when judging emotions, a defect we trace to a lack of spontaneous fixations on the eyes during free viewing of faces. Although SM fails to look normally at the eye region in all facial expressions, her selective impairment in recognizing fear is explained by the fact that the eyes are the most important feature for identifying this emotion. Notably, SM's recognition of fearful faces became entirely normal when she was instructed explicitly to look at the eyes. This finding provides a mechanism to explain the amygdala's role in fear recognition, and points to new approaches for the possible rehabilitation of patients with defective emotion perception.

Patient SM is a 38-yr-old woman whose brain lesion encompasses all nuclei of the amygdala bilaterally, as well as a small portion of the