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COMPARING CLINES ON MOLECULAR AND PHENOTYPIC TRAITS IN HYBRID ZONES: A WINDOW ON TENSION ZONE MODELS

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The study of zones of secondary contact provides insight into the maintenance of reproductive isolation. Tension zone theory supplies powerful tools for assessing how dispersal and selection shape hybrid zones. We present a multimodal analysis of phenotypic clines in conjunction with clines at molecular markers in a hybrid zone between *Larus glaucescens* and *Larus occidentalis*. We developed a new method to analyze simultaneously clines of quantitative traits and molecular data. Low linkage disequilibrium and the lack of coincidence between clines at six microsatellites, a mitochondrial DNA region, and two phenotypic traits indicated introgression. However, the hypothesis of neutral diffusion was rejected based on evidence that all of the clines were concordant and narrower than expected for neutral clines, indicating some indirect selection. The analysis of phenotypic variance gave evidence of restricted phenotypic introgression and together with the bimodal distribution of phenotypes suggested that disruptive selection is acting across the hybrid zone, especially on the coloration of bare parts. Multimodal analysis of phenotypic clines also highlighted a shift between the peak of intermediates and the cline center, left behind by hybrid zone motion. High-resolution analysis of phenotypes distribution thus proved useful for detecting hybrid zone movement even without temporal data.

KEY WORDS: **Cline, Larus, moving hybrid zone, quantitative trait, tension zone theory.**

Hybrid zones are regions in which genetically distinct populations meet and produce hybrids. Hence, they allow research on the consequences of novel epistatic interactions between differentiated genomes. Hybrid zones can take several forms, from large areas of overlap to narrow contact zones or even mosaic zones (Arnold 1992). In one of the most frequent cases, exemplified by the tension zone model, the frequencies of the different alleles or phenotypic traits form clines across the contact zone, maintained by a balance between the homogenizing effect of dispersal and the diversifying effect of selection (Slatkin 1973; Barton and Hewitt 1985). Cline theory provides a powerful conceptual framework to understand the maintenance of hybrid zones and estimate the strength of dispersal and selection from combined measures of cline width and linkage disequilibrium (Barton and Hewitt 1985; Barton and Gale 1993). These indirect estimates are especially useful because they can be based on sample collections across hybrid zones and require no long-term field data (Mallet et al. 1990; Sotka and Palumbi 2006).

Most published studies of hybrid zones make use of neutral markers to measure cline width and linkage disequilibrium

(Kohlmann and Shaw 1991; Dod et al. 1993; Hare and Avise 1996; Porter et al. 1997; MacCallum et al. 1998; Marshall and Sites 2001; Phillips et al. 2004; Steinmetz et al. 2004; Bozikova et al. 2005; Raufaste et al. 2005; Sequeira et al. 2005; Sotka and Palumbi 2006; Yanchukov et al. 2006). Yet, combining neutral markers with variation at phenotypic traits would allow identification of which traits are involved in reproductive isolation or estimating levels of selection (Nürnberger et al. 1995; Brumfield et al. 2001; Bridle and Butlin 2002; Takami and Suzuki 2005; Bridle et al. 2006). Comparing clines of neutral markers with clines of traits known to be under selection also indicates the extent to which the overall genome is under indirect selection because unlinked neutral alleles should pass easily across the clines (neutral diffusion) unless many loci are under strong disruptive selection so that no portion of the genome can introgress (Szymura and Barton 1991; Bridle et al. 2001; Dasmahapatra et al. 2002; Alexandrino et al. 2005; Takami and Suzuki 2005; Grahame et al. 2006). Furthermore, measures of genetic variance in quantitative traits across hybrid zones yield information about the genetic basis of quantitative trait variation, specifically the effective number of loci contributing to the difference between species (Barton and Gale 1993; Sanderson et al. 1992; Nürnberger et al. 1995). The covariance between quantitative traits also provides an estimate of dispersal that is directly comparable to estimates of dispersal obtained using linkage disequilibrium between molecular markers (Mallet et al. 1990; Barton and Gale 1993; Nürnberger et al. 1995; Bridle and Butlin 2002; Dasmahapatra et al. 2002; Alexandrino et al. 2005).

In addition to these classical analyses based on clines of phenotypic traits, a detailed examination of phenotype distribution can improve understanding of selection across hybrid zones. High dispersal and strong selection against hybrids or strong assortative mating result in a bimodal distribution of phenotypic traits (Fig. 1A). This type of contact zone does not show any evidence of extensive hybridization or introgression and is characterized by high linkage disequilibrium and high heterozygote deficiency. This corresponds to a bimodal hybrid zone as defined by Jiggins and Mallet (2000) based on genotypic distributions. If premating isolation or selection is weaker, hints of introgression will be evident, even if no clear "hybrid" phenotypes are observed (Fig. 1B). However, in many hybrid zones, hybrids form a well identifiable group characterized by intermediate allele frequencies and broad genotypic variance due to varying levels of introgression. In this case, the distribution of trait values is better described by a trimodal distribution (Fig. 1C and 1D), two modes for the parental population and one for the intermediates. We suggest that this type of distribution is frequently observed and should not be overlooked. With extensive introgression, the distribution of phenotypes tends toward a unimodal distribution everywhere, as in a hybrid swarm (Fig. 1E). The distribution of phenotypes can also be used to identify movement of a hybrid zone. This is particularly relevant because a common assumption in hybrid zone studies is that populations along the transect have reached migration–selection equilibrium and that clines have been stable for long periods of time (Barton and Hewitt 1985, 1989). This assumption is central to the use of tension zone models and yet remains difficult to assess unless long-term data are available (McDonnell et al. 1978; Moore and Buchanan 1985; Kohlmann and Shaw 1991; Urbanelli et al. 1997; Hafner et al. 1998; Britch et al. 2001; Blum 2002; Dasmahapatra et al. 2002; Buggs 2007).

Despite these advantages, phenotype distributions are rarely considered in hybrid zone studies, partly due to several complications with using clines based on phenotypic traits (Nürnberger et al. 1995). First, nonadditive genetic variance for phenotypic traits may cause distorted phenotypic clines compared to the underlying allelic clines (in both slope and position), because in that case the phenotype is a nonlinear function of underlying allele frequencies. In addition, pleiotropy gives rise to phenotypic covariances in hybrid populations, which can be wrongly interpreted as linkage disequilibrium. Finally, phenotypic plasticity can obscure the relationship between allelic and phenotypic clines.

In this article, we study selection and hybrid zone movement in an avian hybrid zone using a new method to fit clines of molecular genetic and phenotypic data simultaneously and describe the distribution of individual phenotypic trait values. We first investigated the level of reproductive isolation: if epistatic interactions among many loci or genes with large effect prevent introgression in any portion of the genome, we expect high linkage disequilibrium. We also tested for concordance and coincidence in clines of molecular markers and phenotypic traits and compared the observed cline widths with expectations of a neutral diffusion model. We then identified traits under direct selection by comparing phenotype distributions, cline slopes and introgression rates for molecular markers, and phenotypic traits, which we estimated using linkage disequilibrium and phenotypic variance. Finally, we examined the stability of the hybrid zone by assessing the shift between the center of the hybrid zone and the peak of intermediate phenotype frequencies combined with the geographical pattern of linkage disequilibrium.

The Glaucescens–Occidentalis *Hybrid Zone*

The hybrid zone between the glaucous-winged gull *Larus glaucescens* and the western gull *L. occidentalis* in North America is one of the best-studied avian hybrid zones. Much is known about its history and ecology (Dawson 1908; Hoffman et al. 1978; Bell 1996; Bell 1997; Good et al. 2000), but no detailed genetic analysis has been carried out. The glaucous-winged gull breeds from the Gulf of Alaska south through coastal British Columbia

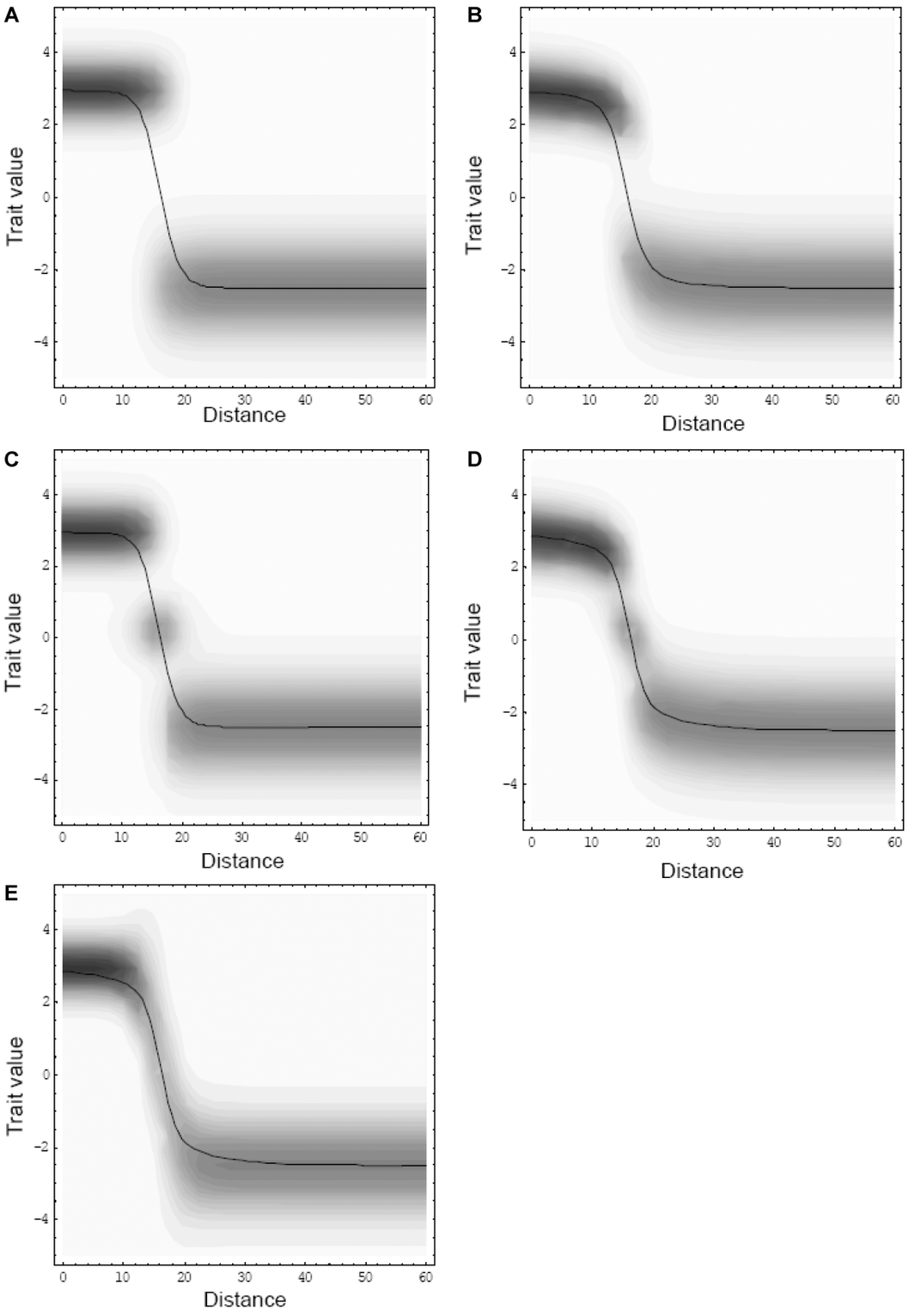

Figure 1. Modeling the distribution of individual trait values to adequately fit morphological clines in a contact zone: (A) The contact zone is simply the admixture of two types without introgression. The black line is the mean trait value across the contact zone and the shading represents the density of probability of individual trait values at a given distance. In this situation, the trait distribution is unimodal away from the contact ($x < 10$ or $x > 20$) but bimodal in between ($10 < x < 20$). This type of contact zone does not show

Figure 1. Continued. any evidence of introgression and would be best described as "bimodal" (Jiggins and Mallet 2000); (B) Trait distribution can show hint of introgression even if no clear "hybrid" phenotype are observed; (C) If some hybrid phenotypes are present, a trimodal contact zone results. This type of situation would be observed if, for instance, some F1 individuals are observed but there is very little introgression in the contact zone (i.e., strong selection against hybrid); (D) With more introgression, F2, F3, and various backcrosses would be present, smoothing out the distribution of individual trait values in the contact zone; (E) With free introgression, the distribution of trait value tends to a unimodal distribution everywhere. This last situation would be best described as "unimodal" in Jim Mallet's typology. The difference between these different situations is not really the shape of the cline on average trait value but the distribution of phenotypes: bimodal (A, B), trimodal (C, D) or unimodal (E).

and Washington to Oregon (AOU 1998). The northern form of the western gull (*L. o. occidentalis*) breeds from islands in Juan de Fuca Strait and the outer Washington coast south to Monterey Bay in central California (AOU 1983). These taxa diverged more than 600,000 years ago (Crochet et al. 2002; Gay et al. 2005) but genetic differentiation between taxa is surprisingly low at allozyme loci (Bell 1996). Interbreeding and phenotypically intermediate individuals were first noted along the Washington and Oregon coastlines at the beginning of the 20th century (Dawson 1908; Pearse 1946; Scott 1971; Hoffman et al. 1978; Weber 1981) and later in a broader zone spanning 500 km from the Queen Charlotte Islands, British Columbia south to Coos Bay, Oregon (Bell 1996). The relative proportion of morphological intermediates at colonies around the center (Grays Harbor, pop. 11, Fig. 2) increased noticeably between 1978 and 1995 (Hoffman et al. 1978; Bell 1997), especially north of the center (Good et al. 2000). Pairs containing at least one phenotypically intermediate bird successfully reproduce (Hoffman et al. 1978) and had greater reproductive success in two populations close to the center of the hybrid zone (8 and 11, Fig. 2). Good et al. (2000) suggested that hybrids could combine adaptive traits from the two parental species, ensuring locally higher breeding success. For example, predation pressure on colonies, especially by bald eagles, is greater in the northern regions than further south (Good 2002). The propensity for *L. glaucescens* and hybrids to choose more vegetated and sheltered nest sites, as opposed to *L. occidentalis*' preference for rocky sites, could create a hybrid advantage, limited to the contact area (Good et al. 2002). Despite hybridization and introgression, previous studies have shown that mate choice remains assortative for morphology (Hoffman et al. 1978; Bell 1997; Good et al. 2000).

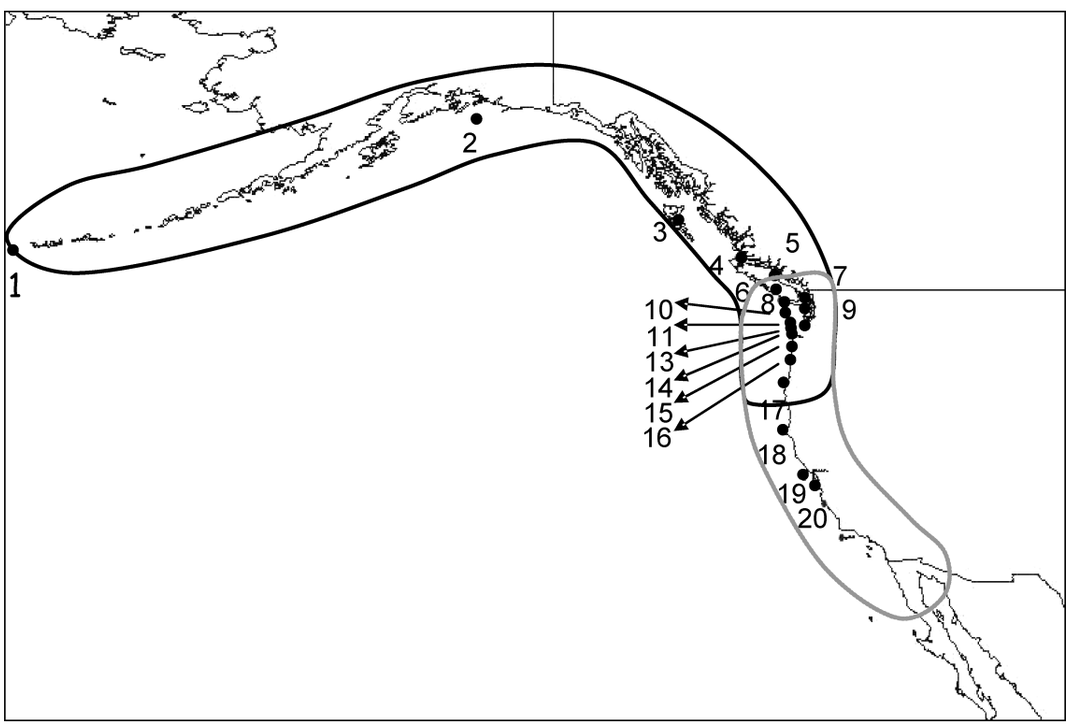

Figure 2. Sampling sites numbered from 1 to 20 across the distributional range of glaucous-winged gull (*Larus glaucescens*, in the North) and western gull (*Larus occidentalis*, in the South).

Material and Methods

SAMPLING ALONG THE HYBRID ZONE AND MEASUREMENT OF PHENOTYPIC TRAITS

Samples and morphological measurements were collected from 1985 to 1990 during the breeding season in coastal Alaska, British Columbia, Washington, Oregon, and California (see Bell 1996 for sampling details). We only used adult specimens collected in the field at nests or from sea or land points near colonies. Voucher specimens are deposited in the Museum of Vertebrate Zoology, University of California Berkeley. Morphological measurements were taken in 19 populations (Fig. 2). Although the range of *L. occidentalis* extends south to Mexico, we decided not to include samples from the populations south of San Francisco Bay because they belong to a different subspecies, *L. occidentalis wymani*. Tissue samples, including liver, heart, kidney, or pectoral muscle were first frozen in liquid nitrogen and then stored at −70°C. Tissue samples were transferred to alcohol for storage prior to DNA extraction and DNA analysis was undertaken on a subsample of 13 populations (Fig. 2). Sample sizes are given in Table 1 and tissue samples are stored in the CEFE (Montpellier) as well as in the Museum of Vertebrate Zoology, UCB.

To characterize the plumage, we measured mantle and primary tip melanism on wing preparations and study skins with a Munsell37-step neutral value scale (Munsell 1971). We measured five traits to describe the coloration of bare parts: bill, iris, pigment of the iris spots, and orbital ring color (measured independently for each eye). Measurements were made on freshly collected specimens using hand-held Munsell color charts (matte finish) from the Munsell Book of Color (Munsell 1976). Each Munsell color is defined by three characters—hue, value, and chroma, from which dominant wavelength, brightness, and excitation purity can be obtained with the aid of conversion tables (Munsell 1968) (see Bell 1996 for additional details on morphological measurements). We thus obtained a total of 15 measurements for the coloration of bare parts for each bird. All individuals were sexed by gonad examination and measured by the same observer (DAB).

We summarized the two sets of highly correlated variables using two independent principal component analyses (using ADE-4 software, Thioulouse et al. 1997): the first on coloration of bare parts (bill, iris, iris spots, and orbital ring color, 15 variables, 527 individuals) and the second on plumage melanism (mantle and primary tip melanism, two variables, 617 individuals). The degree to which coloration of bare parts and plumage melanism are under genetic control is unknown. However, some of the genes involved in determining plumage melanism have been characterized in other species (Theron et al. 2001; Majerus and Mundy 2003) and plasticity of plumage melanism is unreported in gulls. The traits of coloration of bare parts are carotenoïds-based traits and are expected to be relatively plastic (Kristiansen et al. 2006).

Table 1. Sample size for the six microsatellite loci (n), the mitochondrial DNA marker (n_{Dloop}), and the phenotypic traits (n_{morpho}) per population across the hybrid zone between *L. glaucescens* and *L. occidentalis*.

Population	n	n_{Dloop}	n_{morpho}
1	10	7	19
2	8	4	
2b	20	14	34
3			57
4			14
5	23	3	21
6	26		27
7			40
8	35	23	41
9			17
10	18	9	25
11	18	13	58
13			16
14	27	16	27
15			14
16	17	12	18
17	27	12	30
18	18		67
19	26	3	60
20			6

ANALYSIS OF MOLECULAR MARKERS

To extract the total genomic DNA, tissues were digested in 10% Chelex 100 (Bio-rad, Hercules, CA) with 5 μl of proteinase K followed by two 15-min boiling steps following the procedure described in Walsh et al. (1991). Nine microsatellite loci were amplified for 273 individuals from 13 populations. Five loci (HG27, HG25, HG18, HG14, and HG16) were developed for the American herring gull *Larus smithsonianus* (Crochet et al. 2003) and four (K31, K32, K67, and K71) for the black-legged kittiwake *Rissa tridactyla* (Tirard et al. 2002). For HG- loci, the polymerase chain reaction (PCR) was performed in 10 μl reaction volumes containing 2 μl DNA template of variable concentration, 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 2 mM of each dNTP, 0.4 μM of each primer, and 0.1 units Taq DNA polymerase (Eurogentec, Angers, France). The annealing temperature was 58°C for all three HG-loci. K-loci were amplified following the protocol in Tirard et al. (2002). PCR products were visualized using an ABI Prism 310 Genetic analyzer (Applied Biosystems, Foster City, CA). Three loci (HG27, HG25, and K31) displayed one allele close to fixation and no clear differentiation in rare alleles. This could result from variance in the coalescence process or indirect stabilizing selection. As commonly done in hybrid zone studies, these three loci were excluded from further analysis. We estimated the genetic differentiation (F_{ST}) in allopatry (between Alaska pop. 1

and California pop. 19) by the parameter θ (Weir and Cockerham 1984) using GENETIX (Belkhir et al. 1998). The 95% confidence interval was estimated by bootstrapping over loci.

A 674-bp fragment of the mtDNA control region was amplified on a subset of 14 individuals: four individuals from population 2 (GenBank accession numbers: AY615687 to AY615690); two from population 8 (EU780165 and EU780166); one from population 10 (EU780162); four from population 11 (EU780163, EU780164, EU780167, EU780168); and three from population 19 (AY615681 to AY615683). We used the primers L438 and H1561, following procedures described in Gay et al. (2005) and found eight-point mutations that differentiate the species. We designed a PCR-RFLP analysis using the restriction enzyme (BspLU 11I) to identify species-specific mtDNA haplotypes in the PCR-amplified fragment. Two restriction sites were found in the *occidentalis* haplotype (three fragments after digestion), whereas the *glaucescens* haplotype had only one (two fragments). The PCR-RFLP assay was used on 116 individuals in a 25 μl reaction volume containing 1 unit of the restriction enzyme for 1 μg DNA (PCR product) and 2.5 μL reaction buffer. We checked the validity of the designed RFLP marker using the individuals sequenced previously. There was no discrepancy between haplotype sequences and RFLP profiles ($n = 9$).

CLINE FITTING AND ANALYSIS OF MODALITY

We designed a program (Cfit, available at http://www.cefe.cnrs.fr/ecogev/siteGB/CFitpage.htm) to fit clines based on genetic or phenotypic trait data using a simulated annealing algorithm. Both types of clines can be fitted simultaneously to compare their slopes (concordance) and positions (coincidence) and to construct complex hypotheses by constraining some of the parameters involved. The usual cline analysis can be performed by fitting a three-part shape p_x (allele frequency or trait value as a function of distance) with a central sigmoid part and two exponential tails, which is also called a stepped cline (Szymura and Barton 1986), following the equation

$$\begin{cases} 1 - \dfrac{1}{1+e^{-wd_2}} \exp\left(\dfrac{-wt_2(x-c+d_2)}{1+e^{wd_2}}\right) & \text{for x} \le c-d_2 \\ \dfrac{e^{w(x-c)}}{1+e^{w(x-c)}} & \text{for } c-d_2 < \text{x} < \text{c}+d_1 \\ \dfrac{1}{1+e^{-wd_1}} \exp\left(\dfrac{wt_1(x-c-d_1)}{1+e^{wd_1}}\right) & \text{for x} \ge c-d_1 \end{cases} \quad (1)$$

where c and $w/4$ are the center and maximal slope of the cline, respectively; d_1 and d_2, the distance from the center at which the right and left exponential tails start, respectively; t_1 and t_2, the coefficients for the slopes of the right and left tails, respectively. t_1 and t_2 are scaled between 0 and 1 such that the slopes in the tails are constrained to be lower than the slope of the sigmoid at $x = d_1$ or d_2, respectively. This expression considers a negative slope of the cline ($w < 0$).

Because sampling sites in the *glaucescens–occidentalis* hybrid zone were aligned along the coast, we used one dimension clines. Distance between sites was estimated using latitude and longitude, and was cumulative over sites along the coast starting from the southernmost population (population 22, California).

Genetic data

Clines were fitted for alleles frequencies at the six microsatellite loci and the frequency of mtDNA control region haplotypes. To reduce the observed variation in the six microsatellites to two allele systems, we used species-specific compound alleles: for each microsatellite locus, each allele was assigned to a species-specific compound allele (Daguin et al. 2001; Bierne et al. 2003) according to its coordinates on the first axis of a multiple correspondence analysis (MCA) (using GENETIX, Belkhir et al. 1998). Because the collection of specimens was done over a period of 5 years, we verified that the change in allele frequencies was not due to an effect of the year of sampling using a linear model with coordinates on the first axis of the MCA as the dependent variable, year sampled as an explanatory variable, and population as a covariate. We found no significant effect of sampling year ($F_{4,230} = 0.83$; $P = 0.508$). Both species exhibited strong differentiation at all loci analyzed here (see Supporting Fig. S1). Moreover, because the cline shape for shared alleles is flat, a wrong assignment of a shared allele would tend to conservatively flatten the cline at this locus. We fitted all seven genetic clines (six diploid markers and one haploid marker) using a three-stepped cline model. For diploid genetic data, a departure from Hardy–Weinberg proportions (F_{IS}) common to all loci was fitted for each population as implemented in Cfit. We corrected the likelihood for overdispersal of genetic data using the variance inflation factor $\hat{c}$ (Lebreton et al. 1992), which was estimated from the residual deviance of the complete model divided by its residual degrees of freedom.

Phenotypic data

In each parental population, we assume that the quantitative trait z is normally distributed: $N(\mu_{1x}, \sigma_1)$ in the first taxon; $N(\mu_{3x}, \sigma_3)$ in the second taxa, $N(\mu_{2x}, \sigma_2)$ in the hybrids. The means of the three normal distributions (μ_{1x}, μ_{2x}, and μ_{3x}) are allowed to vary with the geographic distance x due to introgression. μ_{2x} is defined as weighted average $k\ \mu_{1x} + (1 - k)\ \mu_{3x}$. In the simplest case of an additive trait, $k = {}^1/_2$. The variances (σ_1, σ_2, σ_3) are constant with x. The distribution of trait values is a weighted sum of these three normal distributions and their relative proportions (p_{1x}, p_{2x}, p_{3x}) vary with x. Each is a function of the mean trait value p_x, which is described by a monotonous cline function (three stepped cline, eq. 1). This model is analogous to the

equations of genotype frequencies at Hardy–Weinberg equilibrium. Similar to the inbreeding coefficient F, the parameter ϕ_p is a function of p_x and allows for fine-tuning the relative proportion of the intermediate mode (see Supporting Fig. S2 for more details).

Cfit was designed to test a set of models corresponding to different distributions of individual trait values (Fig. 1). We compared five models, representing the continuum of phenotype distributions for a similar slope (representing the ratio dispersal/selection) but with decreasing values of dispersal and selection, as presented on Supporting Figure S3: bimodal; bimodal with introgression; trimodal; trimodal with introgression; unimodal (see Supporting Fig. S2 for model parameterization). The different models were compared using AIC (Akaike 1973). We fitted clines to the two phenotypic variables (coloration of bare parts and plumage melanism), using PC1 scores from the two PCAs.

Analyses

We performed a likelihood search for a common center (coincidence) and slope (concordance) of clines at the six microsatellites, the mtDNA control region and the two phenotypic traits. Every fit was checked for convergence by reiterating the fit using different random seeds. Models with regressive levels of constraint either on slope or center were successively fitted for the nine markers and traits simultaneously. Likelihood-ratio tests were used to compare the models for each cline individually and model choice was made at the 5% significance level. Individual clines for which the constrained model was rejected were excluded and fitted independently until the overall model was significantly better than the unconstrained model using AIC (i.e., smaller by more than two AIC points) (Raufaste et al. 2005).

EVIDENCE FOR INTROGRESSION: LINKAGE DISEQUILIBRIUM AND PHENOTYPIC VARIANCE AND COVARIANCE

Genetic admixture between two differentiated populations increases genetic diversity and creates deviations from Hardy–Weinberg proportions. We estimated the number of alleles, the expected heterozygosity (H_e), and the heterozygote deficiency (F_{IS}) in all populations across the transect using GENETIX (Belkhir et al. 1998). Confidence intervals for F_{IS} estimates were obtained by bootstrapping over individuals.

Within a hybrid zone, peripheral parental populations supply parental gene combinations through migration, whereas recombination and segregation in hybrids break them down. An absence of linkage disequilibrium thus indicates extensive introgression and complete recombination of the parental genomes. Multilocus between-genome disequilibrium $K_{0,2}$ (analogous to an average linkage disequilibrium) was estimated using a method developed by Barton (2000) which combines data across loci (http://helios.bto.ed.ac.uk/evolgen/barton/index.html). As recommended, we used "species-specific" compound alleles (as detailed in the cline fitting paragraph) to reduce the noise created by within-species diversity, which is not relevant to the analysis of disequilibria within the hybrid zone. Likelihoods for different nested models were compared using likelihood-ratio tests (Barton 2000). We estimated $K_{0,2}$ in each population and its 95% confidence intervals from the best model. When taken separately, these estimates are not very precise. In addition, drift also generates noise on these values in addition to the uncertainty due to sampling. To overcome these problems, we further fit the linkage disequilibrium pattern across populations. If the frequency variation is a sigmoid, we expect an approximately Gaussian distribution for the pattern of linkage disequilibrium across the hybrid zone. Thus, we used maximum likelihood with normal error on $K_{0,2}$ values to estimate the shape, height (noted D_{max}), and location of this peak.

If the phenotypic variances in parental populations are known, one can estimate the maximum phenotypic variance expected under the hypothesis of complete reproductive isolation (eq. 5b in Barton and Gale 1993). This maximum variance V_{max} corresponds to the admixture of two parental phenotype distributions and equals $(\mu_1 - \mu_3)^2/4 + (\sigma_1^2 + \sigma_3^2)/2$ where μ_1, μ_3, σ^2_1, σ_3^2 are the means and variances of parental populations in allopatry (μ_1 for $x = 0$ and μ_3 for $x = 60$). We compared V_{max} with V_{obs}, the variance calculated from the phenotypic clines, and measured the degree of introgression by the V_{obs}/V_{max} ratio. In addition, the covariance between two phenotypic traits is analogous to a measure of linkage disequilibrium (Barton and Gale 1993). We thus used the same approach as above for $K_{0,2}$ to estimate the shape, height (noted C_{max}), and location of the peak of phenotypic covariance across the hybrid zone.

MOVEMENT OF THE HYBRID ZONE

In a hybrid zone that is moving in one direction, dispersing conspecifics may shift the center of the zone in the direction of movement (c_t on Fig. 5A). In species with long generation times, there is a lag between the formation of hybrid offspring by new dispersers and their return to the breeding population. This results in a deficit of intermediates in the new center (c_t on Fig. 5A) and in the moving front of the hybrid zone, whereas recent intermediates may remain in the original area of the hybrid zone (c_{t-1} on Fig. 5A). Such a shift between the center (c_t on Fig. 5A) and the peak of intermediate (c_{t-1} on Fig. 5A) can be modeled in Cfit by the parameter Φ_P, which modulates the proportion of hybrids similarly to a Hardy–Weinberg deficit (see Supporting Fig. S2).

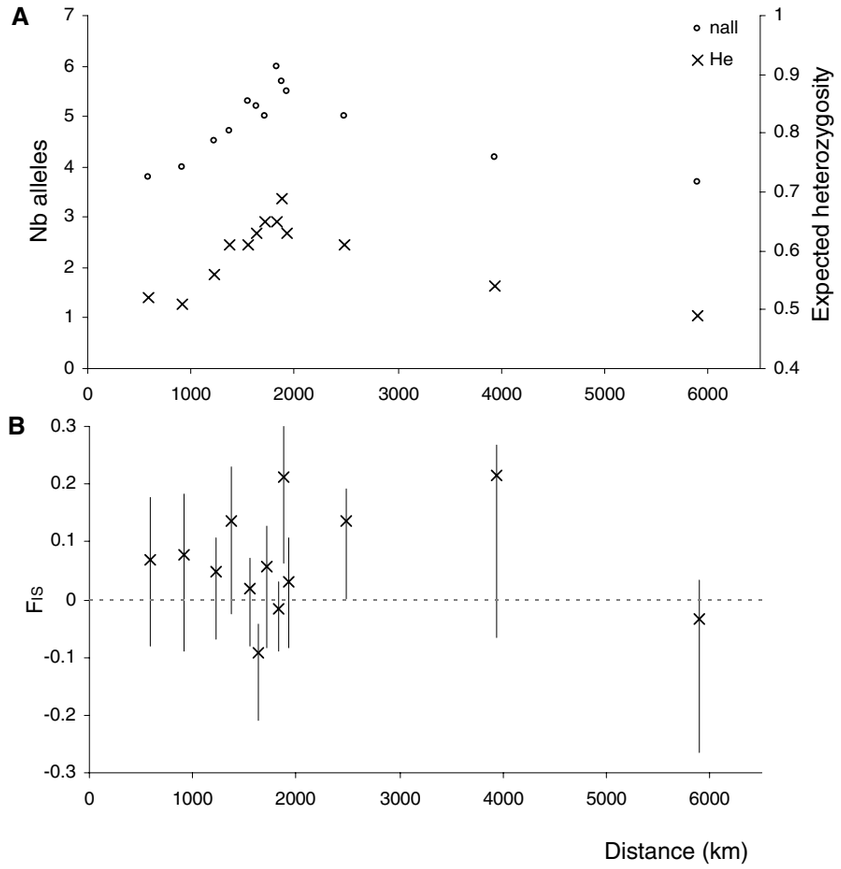

Figure 3. **(A) Number of alleles (n_{all}) and expected heterozygosity (H_e), (B) global F_{IS} for the six microsatellite loci, (C) covariance between coloration of bare parts and the plumage melanism, (D) between genome disequilibrium estimates ($K_{0,2}$, analogous to an average linkage disequilibrium), plotted against the distance along the secondary contact between *L. occidentalis* (South) and *L. glaucescens* (North). The maximum-likelihood fit with normal error on $K_{0,2}$ (D) and phenotypic covariance (C) is represented by the gray curves in (C) and (D). D_{max} and C_{max} are noted on the y-axis. The gray dotted lines stand for the support limits of D (D) and the phenotypic covariance (C). In (D), the black dotted curve stands for $K_{0,2}$ theoretical maximum value, that is, expected if there is no recombination between the two parental genomes (complete reproductive isolation).**

To test if the hybrid zone has moved southwards, we fit Φ_P between the center (c_t) and the north (c_{t-1} on Fig. 5A), where the center would have been located prior to the movement (model a: north in Fig. 5B). We also tested if southward movement coupled with high southwards dispersal results in the presence of new migrants far ahead of the center by constraining Φ_P to form a plateau south of the center (model b: plateauS). For comparison, we also tested if the hybrid zone has moved northward by fitting Φ_P south of the center only (model c: south) and by constraining Φ_P to form a plateau north of the center (model d: plateauN). Finally, in a stable hybrid zone, the peak of intermediates is expected to coincide with the center of the zone. This can be modeled by a flat distribution of Φ_P (model e: constant) or at least a symmetric distribution (model f: symmetric). We compared these six models in Cfit using AIC. Further indication of hybrid zone movement can be obtained by comparing the location in the peak of linkage disequilibrium between markers (or the peak of phenotypic covariance) with the center of the zone as indicated by frequency data.

Results

Populations at the two ends of the transect exhibited large differences in allele frequencies at all molecular marker loci, but only the control region fragment had reached fixation for alternative alleles in *L. glaucescens* and *L. occidentalis* (see Supporting Fig. S1). The six microsatellite loci analyzed showed very low levels of shared polymorphism ($F_{ST} = 0.39$, 95%CI: 0.30–0.52) among which four loci exhibited up to 95% of private alleles. Loci displayed between five and 14 alleles (Fig. 3A). The mean number of alleles and the expected heterozygosity were maximum in the center of the transect (Fig. 3A), which is indicative

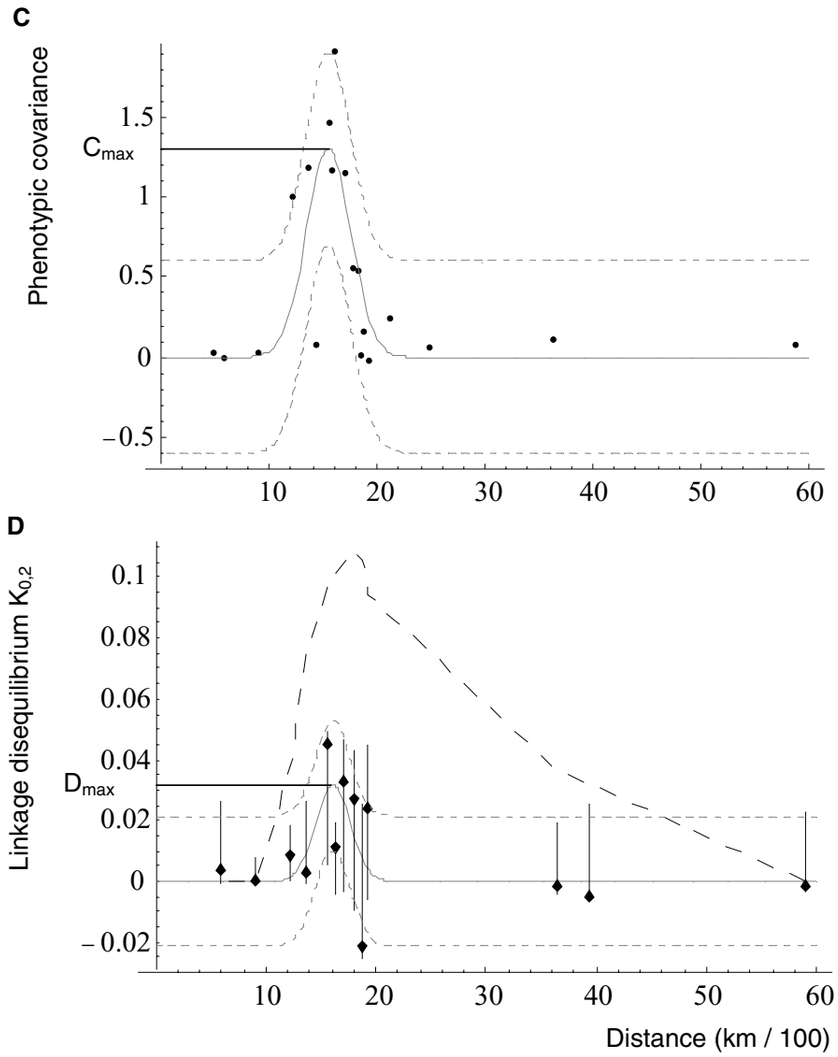

Figure 3. Continued.

of secondary contact. After sequential Bonferoni correction, F_{IS} was significant only in population 6 (Fig. 3B).

INCOMPLETE REPRODUCTIVE ISOLATION

All molecular and phenotypic clines were statistically undistinguishable from a mean 654-km wide cline (defined as the inverse of the maximum slope) but their centers did not coincide (Table 2, Fig. 4). Comparing the models for each cline individually with likelihood-ratio tests found that only three of nine clines could be constrained to share a common center (color, K67 and Dloop, Table 2D). All centers were located between 1349 and 1989 km, that is between populations 5 and 16. The average center (1733 km, support limits: 1664–1802) and the constrained center (coincident model: 1641 km, Table 2) were close to population 10 and 11, in agreement with Bell's results (1997). The best model (common slope but different centers, Table 2B) explained 84.4% of the total deviance on the seven neutral markers and the estimated variance inflation factor $\hat{c}$ was 1.11, indicating a good fit of the data.

If a barrier to gene flow is absent or weak and involves few genes, allelic clines at neutral loci are expected to decay after the secondary contact. In this simple scenario, the width of a neutral cline (w^*) depends only on the dispersal rate (σ) and the number of generations since contact (T) (Endler 1977)

$$w^* = \sqrt{(2\pi)}\,\sigma\sqrt{T}. \qquad (2)$$

L. glaucescens and *L. occidentalis* came into secondary contact at least one century ago (Dawson 1908). Given a generation time in gulls of 10 years (Migot 1992), this secondary contact occurred at least 10 generations ago. We estimated the dispersal distance per generation σ_x from the maximum linkage disequilibrium D_{max}

Table 2. Likelihood search for a common slope (concordance) and center (coincidence) for the nine clines in microsatellites, mtDNA, coloration of bare parts, and plumage melanism (with $k = 0.5$). For each individual locus or trait, the constrained and nonconstrained models were compared using likelihood-ratio tests. Dev stands for deviance and P for the P-value. Traits for which the LRT was highly significant were excluded from the partially constrained model. Width stands for the cline width, defined as 4/w in kilometers; centers are also given in kilometers. The four models were compared globally using AIC (*n* stands for the number of parameters of each model), corrected for overdispersal of the genetic clines ($\hat{c}$). The best model is highlighted in bold.

	(A) No constraint			(B) Slope constrained width = 654.5		(C) Center constrained center = 1641		(D) Three centers constraint	(E) Three centers constraint + slope width = 656
	Width	Center	Dev	Dev	*P*	Dev	*P*	Center	Center
HG18	400	1775	386.4	386.9	0.495	400.3	4.10^{-6}	1796	1792
HG14	524	1795	402.3	402.4	0.797	412.5	0.001	1795	1795
HG16	432	1952	454.4	454.3	1	469.1	2.10^{-5}	1972	1978
K32	1082	1989	371.1	372.9	0.176	398.4	2.10^{-8}	1989	1960
K71	452	1349	379.3	379.5	0.609	395.0	1.10^{-4}	1352	1378
K67	623	1757	452.7	452.8	0.762	455.9	0.104	1614	1614
Dloop	1140	1746	111.5	112.4	0.328	112.4	0.337	1614	1614
plumage	666	1628	724.9	725.0	0.846	734.8	0.002	1602	1810
color	639	1609	1473.8	1473.9	0.708	1475.9	0.146	1614	1614
n			83	75		75		81	73
AIC			4921.9	**4910.1**		5004.5		4921.0	4914.4
AIC $\hat{c}$			4450.6	**4438.3**		4523.4		4449.4	4441.8

$$\sigma^2 = D_{max}\, r / p'^2_{max}(1 + r), \tag{3}$$

where p'_x is the maximal slope of the cline within the hybrid zone (Barton and Gale 1993), and from phenotypic covariances:

$$\sigma^2 = \frac{2rC_{\max}}{z1'_{\max} z2'_{\max}(1 + r)} \tag{4}$$

where $z1'_{\max}$ and $z2'_{\max}$ are the maximal slopes of the clines on the traits z1 and z2, respectively, and $C_{\max}$ the maximum covariance between z1 and z2 (Barton and Gale 1993). The factor $(1 + r)$ is included because the movement probably occurred before sampling (Bell 1996) and thus less dispersal would be required to explain the observed disequilibrium or covariance (Barton and Gale 1993). Note that because the hybrid zone is moving southward (see below), the peak of linkage disequilibrium is expected to be slightly shifted south of the center, causing a transient mismatch between local linkage disequilibrium and local frequency gradient. To circumvent this effect and to estimate dispersal more conservatively, we matched $D_{\max}$ and $p'_{\max}$ in equation (3), even if they do not correspond to the same location (the same comment applies to the covariance method).

The dispersal rate estimated using linkage disequilibrium (σ) was 144 km/generation (support limits: 106–176). Using phenotypic covariances, we estimated $\sigma = 136$ km/generation (support limits: 118–152). This relation does not take into account the uncertainty on the slope estimates and assumes that the covariance between phenotypic traits is created by linkage disequilibrium rather than pleiotropy. We checked this assumption by plotting the covariance against distance. As expected in the absence of pleiotropy, we did not observe any covariance in allopatric populations (Fig. 3C). The support limits account for the uncertainty in estimating $D_{\max}$ and $C_{\max}$. Nevertheless, the two independent estimates are very similar, which further reinforce our confidence in these values.

Taking the mean estimate based on both methods (i.e., 140 km per generation), we find that neutral clines should be 1121-km wide on average after 10 generations of free diffusion. Taking the lower support limits above for dispersal (106 km per generation), we would not expect neutral clines narrower than 838 km today, which is still larger than the width of the clines (654 km) we observe.

INTROGRESSION RATES AND SELECTION

Both coloration of bare parts and plumage were highly divergent in *L. glaucescens* and *L. occidentalis*, as shown by the strong gradient between allopatric populations (Fig. 4). The fit values of the parameter *k* for both phenotypic traits differed from 0.5. This suggests that neither trait is fully additive and indicates that intermediates are more similar to *L. glaucescens* than to *L. occidentalis* (coloration of bare parts: $k = 0.29$; plumage: $k = 0.16$). For both traits, the model "trimodal with introgression" was significantly

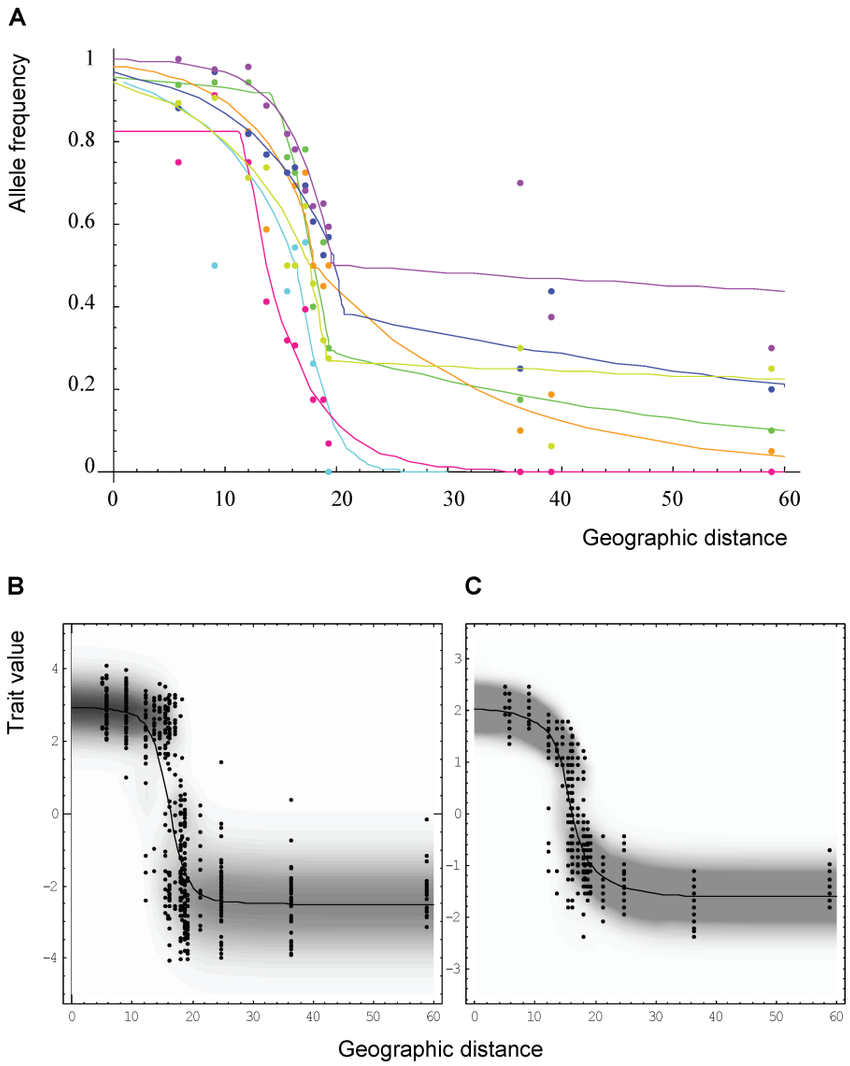

Figure 4. **Cline in the seven molecular markers (A), in coloration of bare parts (B) and plumage melanism (C) along the secondary contact from California (*L. occidentalis* area) to Alaska (*L. glaucescens* area). Lines stand for means and individual values are plotted as dots. For the phenotypic traits, the intensity of dark in background represents the probability density.**

better than any other model (Table 3). In the center, the distribution of phenotypes was bimodal for both traits (Supporting Fig. S5). However, for plumage melanism, the two peaks were very close to each other in the center compared with allopatric populations, which confirms a substantial amount of introgression, especially in *L. occidentalis*.

In a hybrid zone characterized by low dispersal, low selection, and high recombination rate, a very modest increase in linkage disequilibrium and phenotypic variance would be expected close to the center of the zone due to introgression. In Figure 3D, the estimates of the multilocus linkage disequilibrium ($K_{0,2}$) are plotted against distance together with the maximum values expected if reproductive isolation was complete. When each allopatric population is fixed for a diagnostic allele, the maximal linkage disequilibrium in sympatry (corresponding to absence of introgression) is 0.25. In the case of this hybrid zone, allopatric parental populations were polymorphic: the frequency of the "*occidentalis*" synthetic allele varied from 0.18 in the North (population 1) to 0.95 in the South (population 19). The maximal linkage disequilibrium expected in sympatry without introgression was thus ~0.11. We observed a peak of linkage disequilibrium ($D_{\max} = 0.031$, support limits 0.017–0.047) located slightly south compared to the center of the hybrid zone (at 1608 km, support limits 1490–1720) (Fig. 3D). This peak is lower than expected if reproductive isolation was complete (black curve, Fig. 3D). This indicates that there is a large proportion of hybrids in most populations and that they are successfully reproducing, resulting in introgression and recombination of the parental genomes.

Table 3. Comparison of the five hybrid zone models using the AIC, for two quantitative traits (coloration of bare parts and plumage); *n* stands for the number of parameters. The best model is highlighted in bold.

Model	*n*	Coloration of bare parts		Plumage	
		Deviance	AIC	Deviance	AIC
Unimodal	7	1665.3	1679.3	855.1	869.1
Trimodal introgressed					
additive (k = 1/2)	14	**1473.8**	**1501.8**	724.9	752.9
nonadditive	15	1474.1	1504.1	**710.1**	**740.1**
Trimodal	12	1665.3	1689.3	855.1	879.1
Bimodal introgressed	8	1514.0	1530.0	768.3	784.3
Bimodal simple admixture	6	1559.2	1571.2	1163.2	1175.2

Similarly, if a trait is under strong selection, because it is involved in reproductive isolation, it will exhibit a bimodal distribution (Supporting Fig. S3) and therefore will have a large variance in the center of the zone. We observed a large increase of phenotypic variance in the center of the zone for both coloration of bare parts and plumage melanism (Supporting Fig. S4). The degree of introgression was higher for plumage melanism ($V_{obs}/V_{\max} = 0.24; 0.70$ for the coloration of bare parts).

Assuming that the excess variance in the center was primarily due to an increase in the genetic variance at individual loci, we estimated the approximate effective number of loci responsible for differences between coloration of bare parts and plumage melanism to be ~0.7 and ~2.7, respectively (application of the Castle/Wright/Lande method of estimating gene numbers, Barton and Gale 1993). This further assumes that the differences are diagnostic, and that all loci have the same effect and the same allele frequencies.

MOVEMENT OF THE HYBRID ZONE

For the coloration of bare parts, the "plateauS" model was significantly better (model b, Table 4). This suggests that there is a relative excess of intermediates north of the center (c_{t-1} in Fig. 5A, in the range of *L. glaucescens*) and a large deficit in the center (c_t) and further south (in the range of *L. occidentalis*) (Fig. 5C). Indeed, the hybrid zone is bimodal for the coloration of bare parts over a distance of about 300 km. For plumage melanism, the best model was to fit Φ_P north of the center only (model a, Table 4). This also implies that there is a relative excess of intermediates north of the center (c_{t-1}) and a relative deficit of intermediates in the center (c_t, Fig. 5C). Contrary to the coloration of bare parts, plumage melanism does not have a bimodal distribution south of the center. Because the introgression rate is higher for plumage melanism, recent migrants are also probably introgressed, which could attenuate the bimodal distribution further south. The shape of Φ_P for both phenotypic traits is very similar to the shape expected if the hybrid zone is moving southwards (Fig. 5B). The southward movement is also noticeable when we directly compare the observed distribution of both phenotypes and genotypes (Supporting Fig. S5) with the distribution expected following a movement, as outlined below the cline on Figure 5A. This is also consistent with the southward shift in linkage disequilibrium (peak located at 1608 km, support limits: 1490–1720) and phenotypic covariance (peak located at 1547 km, support limits: 1467–1618), compared to the overall center of the hybrid zone estimated from frequency data on all molecular markers and phenotypic traits (located 1733 km, support limits: 1664–1802).

Table 4. Test of the movement of the hybrid zone by comparing models with different constraints on the proportion of hybrids Φ_P: Φ_P fitted north only (model a); Φ_P plateauN (model b); Φ_P fitted south only (model c); Φ_P plateauS (model d); Φ_P constant (model e) and Φ_P symmetric (model f). Dev and np stand for the deviance and the number of parameters of each model. The best model is highlighted in bold.

		movement southward		movement northward		no movement	
		a north	b plateauN	c south	d plateauS	e constant	f symmetric
Plumage	dev	714.5	721.6	717.1	720.2	721.9	720.0
	np	12	12	12	12	11	13
	AIC	**738.5**	745.6	741.1	744.2	743.9	746.0
Color	dev	1511.9	1477.1	1495.0	1499.7	1501.2	1491.6
	np	11	11	11	11	10	12
	AIC	1533.9	**1499.1**	1517.0	1521.7	1521.2	1515.6
	np	23	23	23	23	21	25
Total	AIC	2272.5	2244.7	2258.1	2265.9	2265.1	2261.5

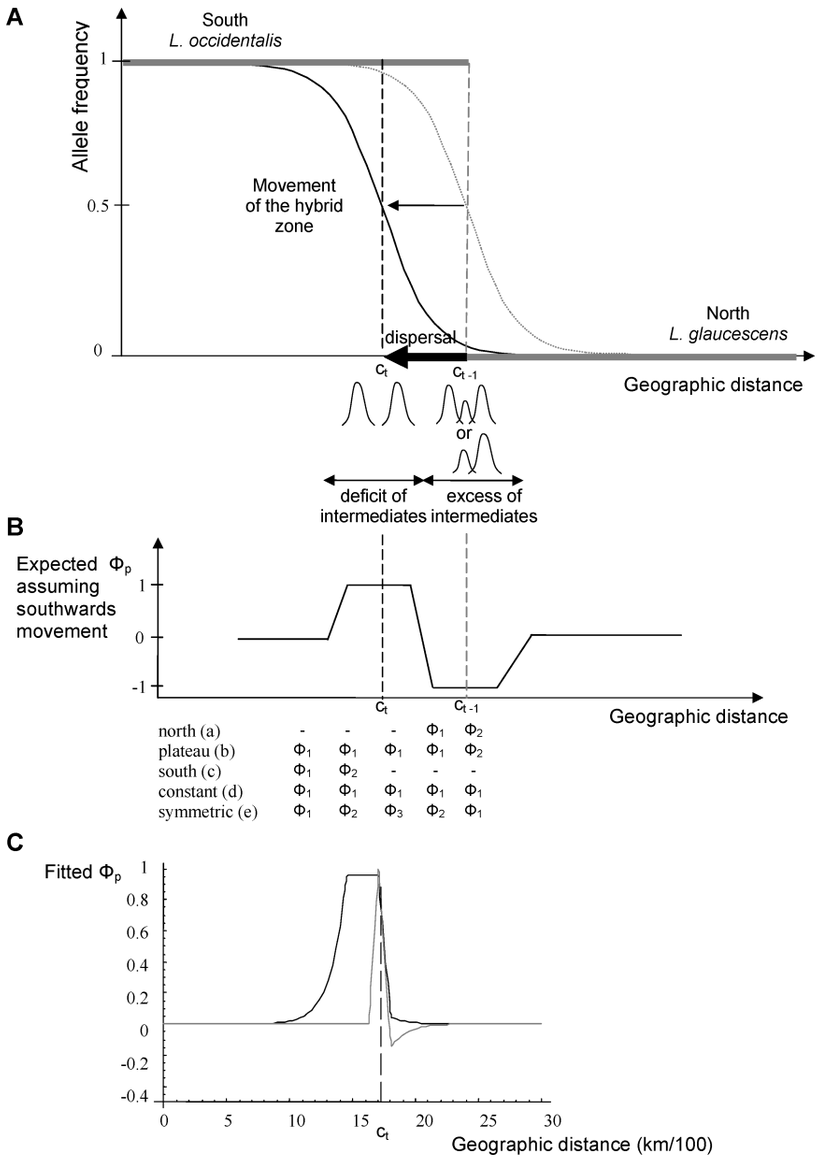

Figure 5. (A) Schematic representation of a movement of the zone that could create the deficit of intermediate observed in the center and an excess of intermediates north of the center. The gray horizontal lines stand for the distribution of both parental taxa before movement. The black arrow represents the recent dispersal of *L. glaucescens* southward. Such dispersal would have moved the cline southward, leaving a peak of intermediates behind (located at the previous center, gray dashed line). (B) Expected values of Φ_p assuming a movement of the hybrid zone southward, resulting in a large bimodal area in the center and an excess of intermediates north of the center and table describing the parameters is fitted for each model (see text). (C) Fitted estimates of Φ_p for the coloration of bare parts (in black) and the plumage melanism (in gray) for the best models. The average center is indicated by the black dashed line.

Discussion

INCOMPLETE REPRODUCTIVE ISOLATION

The combined analysis of clines of molecular markers and phenotypic traits confirmed that reproductive isolation between *L. glaucescens* and *L. occidentalis* is incomplete, in agreement with previous studies (Hoffman et al. 1978; Bell 1996). First, both genetic linkage disequilibrium (Fig. 3D) and phenotypic variance (Supporting Fig. S4) were significantly lower than expected if reproductive isolation was complete. Second, the trimodal distribution of both phenotypic traits clearly indicates the presence of intermediates in the contact zone. Hence, both molecular markers and phenotypic data confirm that reproductive isolation between *L. glaucescens* and *L. occidentalis* is incomplete.

Allele frequencies for nuclear markers as well as for a mitochondrial marker and two phenotypic traits nevertheless formed concordant stepped clines. We also found a peak of linkage disequilibrium and phenotypic covariance slightly south of the center of the zone (Fig. 3C, D). This suggests that there are parental genotypes in the center of the hybrid zone. This is confirmed by microsatellite data (assignment methods, results not shown). Given that neutral clines should be 1121-km wide on average (and not narrower than 838 km) after 10 generations of free diffusion, it is very likely that some indirect selection is acting on the molecular markers studied here. This is not surprising given that (1) there is certainly disruptive selection at least on the coloration of bare parts (see next paragraph), (2) the hybrid zone is moving (indicating that selection favors one taxon over another, see below).

A potential source of uncertainty comes from our estimates of dispersal (144 and 136 km/generation depending on the method). They are within the range of or slightly higher than known estimates of dispersal in gulls (Cramps and Simmons 1983; Coulson 1991; Oro and Pradel 1999), but demographic methods frequently underestimate long-distance dispersal (Jones et al. 1981; Moore and Dolbeer 1989; Crochet 1996; Phillips et al. 2004). Indirect methods may nevertheless yield biased estimates of σ if the distribution of parent–offspring distance is leptokurtic (Rousset 2004). However, we obtained similar yet independent estimates from the genetic and phenotypic data, which increases our confidence in the dispersal estimates. Using linkage disequilibrium (D) to infer dispersal is a method that has been developed with the stable tension zone in mind. The key approximation, however, is that D quasi-equilibrates fast compared to allelic frequency changes (Barton and Gale 1993; Nagylaki 1993). In the case of a nonstable hybrid zone, this method may still apply provided that the markers are loosely linked and that clines move slowly enough. In particular, a movement of the zone may inflate D locally compared to the underlying frequency gradients. Here, we observe that D and the phenotypic covariance both peak south of the center of the zone (1608 and 1547 km, respectively), which is consistent with a southward movement of the hybrid zone. To correct for this movement, we estimated dispersal conservatively by matching the maximum D and the maximum gradients. The tension zone model also assumes that dispersal can be summarized by a Gaussian kernel. This may be problematic given that cline slope may differ depending on the detail of dispersal (e.g., if it is very leptokurtic or inhomogeneous, Rousset 2004) and this assumption is difficult to check. Another source of uncertainty comes from the estimate of time since secondary contact (T), which is based on estimates of generation time and field observations from the literature (Dawson 1908; Migot 1992). T is more likely to be underestimated, which is conservative to test for neutral diffusion and uncertainty is small because generation time varies in a restricted scale. It should thus have limited effect on the estimation of the slope expected under neutral diffusion. This supports the idea that clinal variation in the contact zone between *L. glaucescens* and *L. occidentalis* is not shaped by neutral diffusion and that indirect selection may be acting on the molecular neutral markers used in this study.

DIRECT SELECTION ON THE COLORATION OF BARE PARTS

For both sets of phenotypic traits, the model "trimodal with introgression" fits better than the unimodal model (Table 3), which suggests that disruptive selection is acting across the zone (see Supporting Fig. S3D). Indirect selection could explain the limited increase in phenotypic variance in the center of the zone (see Supporting Figs. S4 and S5) similar to the increase of linkage disequilibrium. However, the strong increase in variance for coloration of bare parts implies that moderate disruptive selection is acting directly on this trait, although we did not detect a significantly smaller width for this cline (the point estimate is 639 km, Table 2). This may be due to the strong heterogeneity among loci for cline width (from 400 to 1140 km, Table 2).

Provided that the nongenetic variance and the intraspecific genetic variance are small, the phenotypic differences observed may be due to a small number of loci of large effect. As mentioned earlier, this hypothesis might not apply for the coloration of bare parts, because carotenoids-based traits are probably highly plastic. Because we do not have data on heritability, our estimate of the effective number of loci involved should be considered as a minimum value. Disruptive selection might result from intrinsic lower fitness of hybrids (endogenous postmating barrier) or environment-dependent selective pressures (exogenous selection). These two scenarios are difficult to tell apart based on the shape of the cline (Kruuk et al. 1999). Moreover, the bimodal distribution of the coloration of bare parts in the center of the hybrid zone suggests the existence of premating barriers, for example assortative mate choice (Jiggins and Mallet 2000). It has been previously suggested that the coloration of bare parts may be involved in mate choice in seabirds (Pierotti 1987). Further experimental testing is needed to confirm that traits like orbital ring and bill color are involved in mate choice and contribute to assortative mating. Introgression of both phenotypic and molecular markers appears to be slowed down by selection in this hybrid zone. A reduction of gene flow for the coloration of bare parts compared to the introgression rate for neutral markers was found in another hybrid zone between gull species in Europe (Gay et al. 2007), despite a much lower genetic divergence between the hybridizing taxa. This reinforces the idea that coloration of bare parts plays a role in mate choice and could be involved in premating isolation between large gull species.

MOVEMENT OF THE HYBRID ZONE

Using the method presented here to estimate the center of a cline on quantitative traits and model the distribution of phenotypes independently enabled us to detect a southward movement of the hybrid zone. The bimodal distribution detected in the current center (c_t) and further south reflects the presence of *L. glaucescens* individuals, likely recent dispersers, who may not have produced offspring old enough to have returned to the colony to breed yet. Extensive introgression of *L. occidentalis* individuals by *L. glaucescens* north of the center (in place of the old center, c_{t-1} on Fig. 5A) created a relative excess of intermediates.

This relative excess of intermediates on one side of a cline and deficit on the other could alternatively be explained by a complex selective scenario. However, without further evidence we believe that the simple movement hypothesis is sufficient to account for the observed pattern. Moreover, the hypothesis of movement of the hybrid zone is supported by several other lines of evidence. First, field observations suggest that *L. glaucescens* breeds as far south as population 17, whereas *L. occidentalis* only reaches population 8 (Bell 1996). This is confirmed by our genetic data, which show that individuals assigned as "pure" *L. glaucescens* breed as far south as population 16 whereas "pure" *L. occidentalis* do not spread north of the center (population 10) (assignment using microsatellite data, results not shown). Second, the fit of D and phenotypic covariance both peak south of the center of the zone (1608 and 1547 km, respectively), which is consistent with a southward movement of the hybrid zone. Third, a movement of the hybrid zone could explain why intermediates look more like *L. glaucescens* for both phenotypic traits ($k < 0.5$, but significant for plumage melanism only, Table 3). This could result from asymmetric introgression if there is an excess of backcrosses to *L. glaucescens* as expected if *L. glaucescens* is expanding southwards. Alternatively, this could be due to dominance or epistasis distorting the phenotypic cline compared to the underlying allelic clines.

Different factors can drive hybrid zone movement (Barton and Hewitt 1985; Blum 2002; Buggs 2007), such as a selective advantage of one phenotype in a given environment (Goodman et al. 1999), dominance drive (Blum 2002), asymmetric migration (Goodisman et al. 1998), competition (Hafner et al. 1998; Dasmahapatra et al. 2002), asymmetric hybridization (Bronson et al. 2003; Buggs and Pannell 2006), hybrid fitness (Klingenberg et al. 2000), or human activity and climate change (Britch et al. 2001). The southward movement of the *L. glaucescens* × *L. occidentalis* hybrid zone suggests higher competitive abilities or a selective advantage of *L. glaucescens*. Field observations report a higher breeding success of *L. glaucescens* compared to *L. occidentalis* or intermediates (Good et al. 2000, larger clutch size, hatching, and fledging success) although the results seem to be variable depending on the population studied and possibly the presence or absence of El Nino-like conditions in the Pacific Northwest (Bell 1996, 1997). Predation could limit the spread of *L. occidentalis* northwards, because there is no selective pressure to nest in vegetated habitat in its original distribution range (Good et al. 2000). Indeed, the number of intermediate phenotypes was found to increase in the center (population 11, +25% between 1989 and 1995) to the detriment of *L. occidentalis* phenotypes due to the latter's low breeding success compared to *L. glaucescens* or intermediates (Good et al. 2000).

In a hybrid zone maintained by exogenous selection, clines will be rooted to the environmental boundary (Leache and Cole 2007). Because the environment can be highly heterogeneous, most tension zones are thought to be trapped in regions of low density, which act as sinks for migration (Barton and Hewitt 1985, 1989). For such reasons, hybrid zone movement has long been considered rare. However, in organisms able to disperse very long distances, such as gulls, physical barriers are less prone to limit the movement of hybrid zones. Indeed, moving hybrid zones have been detected in various taxa using successive sampling. A total of 23 clear examples were documented in a recent review (Buggs 2007), to which we suggest adding three more (Moore and Buchanan 1985; Kohlmann and Shaw 1991; Urbanelli et al. 1997). A number of studies have also inferred hybrid zone movement from observed asymmetrical introgression across hybrid zones, assuming that the mitochondrial or other genetic wake is a relict of that movement (Parson et al. 1993, and see Buggs (2007) for another 16 examples). However, among the studies in which movement was unambiguously demonstrated by resampling, some found a mitochondrial wake (Leache and Cole 2007) whereas others did not (Dasmahapatra et al. 2002). The method presented here to fit clines on quantitative traits offers an alternative to detect hybrid zone movement using the shift between the center of the zone and the peak of intermediates.

Hybrid zone movement can result in the invasion of one taxon over the distributional range of another (Woodruff and Gould 1987; Carney et al. 2000; Perry et al. 2001; Rohwer et al. 2001). Hybrid zone movement is also of key importance in the debate about adaptation via "shifting balance" (Wright 1982; Coyne et al. 2000), because it could allow a new adaptive peak to spread to other populations (Rouhani and Barton 1987; Barton 1992; Kondrashov 1992). Finally, the tension zone model assumes stable hybrid zones, at equilibrium between dispersal and selection. One way to check this is to obtain data over several years. However, as we showed in this article, a snapshot may be enough to detect a possible movement of a hybrid zone using the distribution of phenotypes within the zone. Because it is not always possible to follow hybrid zone through time, it is not always possible to tell whether the tension zone has reached the dispersal/selection equilibrium or is still moving. In this context, utilizing the method we have outlined here may prove particularly fruitful.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Allele frequency tables for the six microsatellite loci and the mitochondrial DNA fragment.

Figure S2. Adjusting clines on quantitative traits.

Figure S3. Relation between the slope of a cline w, selection s and dispersal σ in a tension zone model ($w^2 = 8\,\sigma^2/s$, Barton and Gale 1993).

Figure S4. Distribution of phenotypic variance across the *L. glaucescens – L. occidentalis* hybrid zone (curve: fitted; dots: observed), for two quantitative traits: coloration of bare parts (a) and plumage (b).

Figure S5. Composition of populations represented by frequency distributions of phenotypes along the zone of secondary contact from *L. occidentalis* (top left) to *L. glaucescens* (bottom right) for the coloration of bare parts, the plumage melanism (distributions fitted by C-Fit) and the genotype (from AFC, results not shown).

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