Gene flow and genetic admixture across a secondary contact zone between two divergent lineages of the Eurasian Green woodpecker Picusviridis

J.-M. Pons, C. Masson, G. Olioso, J. Fuchs

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Pons J.-M.*, Masson C.†,Olioso G.‡ and Fuchs J*.

*Département Systématique et Evolution, UMR7205 Institut de Systématique, Evolution, Biodiversité CNRS MNHN UPMC EPHE, Sorbonne Universités, Muséum National d'Histoire Naturelle, CP51, 57 rue Cuvier, F-75231 Paris Cedex 05, France

†63 rue des jonquilles 91210 Draveil

‡Georges Olioso, 248 rue de l'industrie, Port-le-Nouvelle, 11210, France.

*Corresponding author: jean-marc.pons@mnhn.fr

running title: gene flow across *P. viridis* contact zone

SUMMARY

Secondary contact zones are efficient natural systems to measure genetic differentiation and gene flow and thus provide good opportunity to assess the level of reproductive isolation between divergent evolutionary lineages. In this study, we used ten Z-linked and nine autosomal loci from seven chromosomes and twenty males to evaluate gene flow across a secondary contact zone between two mitochondrial lineages of the Eurasian Green Woodpecker (*Picus viridis*), that diverged around one million years ago. One lineage (*sharpei*) is distributed throughout the Iberian Peninsulawhereas the other one (*viridis*) is widespread acrossthe Western Palearctic. These two lineages form a secondary contact zone in southern France. Formerly treated as two subspecies of *Picus viridis*, several authors have recently proposed to assign a specific rank to *P. v. sharpei* and *P. v. viridis*. Our results indicate no introgression of nuclear loci in allopatric populationslocated on both sides ofthe contact zone which thus actsas an efficient barrier to gene flow. All males sampledwithin the contact zone and one male sampled near its eastern border were slightly admixed revealing that reproductive isolation between *sharpei* and *viridis* is not completely achieved.In accordance with the geographical range of each lineage, the two admixed males sampled near the western border of the contact zone harboured a large proportion of *sharpei* alleles whereas admixed males sampled eastwardlynear the Rhone Valley had a high proportion of *viridis* alleles. Overall our results bring further support to consider *sharpei* and *viridis* as two biological species.

KEYWORDS

Hybrid zone, introgression, clines, Z-linked loci,autosomal loci, gene flow, speciation, *Picusviridis*.

ACKNOWLEDGEMENTS

We are very grateful to E. Garcia Franquesa and J. Quesada (Museu de Ciences Naturals, Barcelona), G. Boano (Museo Civico di Storia Naturale di Carmagnola, Carmagnola), E.Borgo (Museo di Storia Naturale di Genova, Genova), O. Llama Palacios and I. Rey Fraile (Museo Nacional de Ciencas Naturales, Madrid) for sending us tissue samples. Sampling in southern France was legally done under a CRBPO (Muséum national d'Histoire naturelle) program directed by Georges Olioso. We thank Liviu Parau and one anonymous referee for their helpful comments. The laboratory work was supported by the 'Service de Systématique Moléculaire' (UMS 2700 OMSI, MNHN) and we thank C. Bonillo, D. Gey, J. Lambourdière (UMS 2700) for their help. We also thank Ben Warren for his suggestions and help with English. This work was funded by the UMR 7205, Institut de Systématique, Evolution, Biodiversité.

INTRODUCTION

Zones of secondary contacts are very interesting for evolutionary biologists because they provide unique opportunities to assess the level of reproductive isolation between closely related taxa that have recently differentiated (Barton and Hewitt 1985). The limit between intraspecific population divergence and interspecific divergence is often difficult to hypothesize, especially when taxa are not in geographical contact. When taxa that have evolved in allopatry meet each other in a secondary contact zone, it becomes possible to safelytest their species status. Depending on the evolutionary history of both taxa and the stage of completion of the speciation process, several scenarios are possible when they form a secondary contact zone. If strong reproductive barriers have evolved in allopatry, both taxa will not be able to form hybrids,and thus to mix their genomes when they come into geographical contact. By contrast, if reproductive isolation is absent, bidirectional widespread introgression erasesgeneticdivergence that evolved in allopatry, and eventually results in the complete merging of parental genomes and disappearance of the contact zone(Endler 1977). In case of recent divergence, however, reproductive barriers are often incomplete allowing taxa to hybridize to a more or less extent (Barton and Hewitt 1985).Depending on the efficiency of pre-zygotic (assortative mating) and post-zygotic reproductivebarriers (selection against hybrids), we may expect that the movement of genes across the contact zoneis more or less slowed down (Hewitt 1988).

By analyzing the geographical distribution of cytochrome b and Z-linked BRM intron-15 alleles in southern France, Spain and Europe, Pons et al. (2011)concluded that the Iberian and the European lineages of the Eurasian Green Woodpecker (*Picus viridis*, Linneaus 1758) came into secondary contact along thePyrenees chain and in the Languedoc-Roussillon region along the Mediterranean coast.Pons et al. (2011) using molecular datingsuggested a mid-Pleistocenesplit (~1 million years ago) between these two lineages, of which one isrestricted

115 to the Iberian Peninsula and southern France and the other is found over the rest of Europe up
116 to western Russia. According to Pons et al. (2011), allopatric divergence resulting from the
117 Pleistocene climatic oscillations isthe most probable scenario that would explain genetic
118 divergence of the Iberian population from the other one found elsewhere in Europe.
119 Both taxa differ by several plumage characters including the color of the face which is grey in
120 the Iberian Green woodpecker (*sharpei* lineage) and black in the Eurasian Green woodpecker
121 (*viridis* lineage) (Winkler and Christie 2019;Olioso and Pons 2011). Individuals with
122 intermediate plumage were mentioned at the beginning of the 20th century in the Languedoc-
123 Roussillon region (Jouard 1928) and are still currently observed (Olioso and Pons 2011). The
124 taxonomic treatment of the Iberian lineage varies according to taxonomic authorities and is
125 considered either as a species (*Picus sharpei,* H. Saunders, 1872) (Perktas et al. 2011; Gill and
126 Donsker 2018; Del Hoyo et al. 2019) or a subspecies (*Picus viridis sharpei*) (Dickinson and
127 Moore 2013).
128 In this study, we assess gene flow and genetic introgression between *sharpei* and *viridis*
129 across the Mediterranean secondary contact zone withten Z-linked loci and nine autosomal
130 loci using Bayesian inference of genetic population structure and spatial variation in alleles'
131 frequencies.
132 Our primary aims are:(1) to test whether the secondary contact zone acts as an efficient barrier
133 to the eastward moving of *sharpei* alleles and the westward moving of *viridis* alleles and (2)
134 to assess the level of genetic admixture within the contact zone.
135 METHODS
136 ***Sampling design***
137 Two allopatric populations were sampled for each lineage and one population was sampled
138 across the contact zone in southern France. According to their distances from the contact
139 zone, populations which were oriented along a South-West/North-East transect were referred

as close allopatric populations, populations 2 and 4 (Catalonia and Provence, Fig. 1), or distant allopatry, populations 1 and 5 (Central Spain and North-West Italy, Fig. 1). Within each population, four males were chosen at different localities that were several kilometers apart (Fig. 1, Table S1). The studied contact zone (population 3) reported on Fig. 1 has been delimited after Pons et al. (2011) and Olioso and Pons (2011) as the area where the two mitochondrial lineages overlap and where birds with intermediate plumage are encountered. Across the contact zone, four males were sampled so as to be widely distributed from the western side of the contact zoneup to the eastern border (Fig. 1). Tissues (muscles) from allopatric populations 1, 2 and 5 were obtained through loans from the Museo Nacional de Ciencias Naturale (Madrid), Museu de Ciències Naturals de Barcelona, Museo di Storia Naturale di Genova, and Museo Civico di storia naturale di Carmagnola (Table S1). Information concerning the sampled individuals, localities and tissue numbers is reported in Table S1.Pictures showing plumage variation of three adult males sampled across the contact zone and one male from population 4 are given in Fig. S1. Tissue samples (feathers) from southern France were collected from birds caught during the breeding season as adults (n=6) or fledglings (n=2).Feathers samplingand bird ringing waslegally performed as part of the "Centre de Recherches sur la Biologie des Populations d'Oiseaux" ringing program (Muséum national d'Histoire naturelle) directed by one of the authors (G. Olioso).

Laboratory work

Ten Z-linked loci and nine autosomal loci were sequenced for the four males from each population. We only included male individuals in our samples because males are the homogametic sex in birds (ZZ) whereas females are ZW. Hence eight alleles per locus were sampled for each population.Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) andused for PCR amplification and sequencing of nineteen loci. Primer sequences are listed inTable S2.The thermocycling conditions were standard and included a hot start at

94 °C, an initial denaturation at 94 °C for 3 min, followed by 35–40 cycles at 94 °C for 40 s, 52–60 °C for 30 s, and 72 °C for 30–60 s, completed by a finalextension at 72 °C for 5 min.PCR products were sent to Eurofins Genomics (Ebersberg, Germany) for purification and Sanger sequencing.The assignment of each locus to its respective chromosome and position of Z loci along the Z chromosome were based on the *Gallus gallus* genome and position of the analysed loci on the *Picoides pubescens* genome scaffolds are given in Table 1. All sequences have been deposited in Genbank with the Accession Numbers XXX.In addition, we sequenced afragment of the cytochrome b gene (cyt b, 427 bp) to check the mitochondrial lineage of each bird.

Genetic diversity, genetic divergence and structure analyses

We used PHASE v2.1.1 (Stephens et al. 2001), as implemented in DNASP 5.0 (Librado and Rozas 2009), to infer the alleles for each nuclear locus.We tested for recombination within each nuclear locus by using the SBP (Single Break Point) algorithm (Kosakovsky Pond et al. 2006) as implemented on the DATAMONKEY webserver (www.datamonkey.org; Delport et al. 2010).

We did not detect any evidence of recombination in the nuclear loci using the Single Break Point algorithms. The numbers of polymorphic sites and of segregating sites were estimated withDNAsp (Librado and Rozas2009). Nucleotide diversity (π) and Watterson's theta (Θ) were estimated with DNASP for each lineage. For each locus,net nucleotide divergence (Da) and fixation index (Fst)between allopatric populations (*sharpei* populations 1 and 2; *viridis* populations 4 and 5 – eight males each)were calculated to estimate the level of genetic divergence between *viridis* and *sharpei* lineages.

Genotypic data were also analyzed in STRUCTURE 2.2.3 (Pritchard et al. 2008) to group individuals into populations without a priori knowledge on putative populations.STRUCTURE uses a Bayesian model-based clustering method to infer

population structure and to assign individuals probabilistically to a set of populations (K) based on allele frequencies across loci. Based on the estimated posterior probability for each run and for each value of K, STRUCTURE estimates the likelihood of the data. We assumed an admixture model with correlated allele frequencies and let alpha vary among populations. We ran $5*10^6$ iterations (burnin: $5*10^5$ iterations) from K = 1 to K = 5. For each K, we ran 10 analyses. The number of clusters (populations) was estimated using Evanno's ΔK (Evanno et al. 2005), as calculated using STRUCTURE HARVESTER v 0.6.8 (Earl and vonHoldt 2011). The output files from STRUCTURE HARVESTER were then used in CLUMPP v1.1.2 (Jakobson and Rosenberg 2007) to perform label switching and obtain the best estimate of the membership coefficient. Plots were visualised in DISTRUCT v1.1 (Rosenberg 2004).

We used TCS 1.21 (Clementet al.,2000) to reconstruct a 95% statistical parsimonynetwork for each locus.

Allele frequency clines

Our five populations were situated along a south-west/north-east transect that spans approximately 1500 kms. We transformed the two-dimensional information of each individual (latitude-longitude) into a single geographic dimension (e.g. Whibley et al. 2006) using ArcGis 10.2 and QGis 2.2.0-Valmiera. Each individual was projected orthogonally along a transect starting in central Spainand ending innorth-west Italy. Average distancesbetween populationswere calculated with reference to the population 1(Central Spain)which was selected as the starting point of the transect.

We built clines to visualize changes in allele frequencies across space. Allele frequencies of 19 loci were obtained with GenoDive V2.0 (Meirmans and Van Tienderen 2004). Clines were built with the package *hzar* (Derryberry et al. 2014) that provides functions for fitting molecular genetic data from hybrid zones to classic equilibrium cline models using the Metropolis–Hastings Markov chain Monte Carlo (MCMC) algorithm. Cline centre and cline

width estimates and 95% confidence intervals were calculated for each loci. Cline models were then compared to a null model under which allele frequencies are independent from geographical location using the Akaike Information Criterium corrected for small sample sizes. We used a Kruskal-Wallis Chi-square test to compare the mean cline parameters obtained for autosomal loci and Z-linked loci respectively.

RESULTS

We sequenced 9 autosomal and 10 Z-linked loci. Among these19 loci, one Z-linked locus (KIF24; 233 bp) was monomorphic and thus was not included in the analyses. Hence, we obtainedalleles sequences from 40 autosomal chromosomes and 40 Z chromosomes for all loci but one. Only thirty-eight chromosomes were sampled for the autosomal locus 13336 located on chromosome 7 as we could not amplify this locus for one individual.

Intra-subspecific polymorphism

We estimated intra-subspecific polymorphism using the distant and the close allopatric populations (*sharpei*pop1, pop2, *viridis*pop4, pop5) anddid not detect introgression in either case.Levels of genetic variation estimated with π and θ were quite low for both subspecies,averagevalues varying from 0.0003 to 0.0007 and from 0.0004 to 0.001, respectively(Table 1). There was no statistical difference in nucleotide diversity either between both lineages or between Z-linked and autosomal loci (all Mann-Whitney tests not significant at the 0.05 level).

Geneticdifferentiation between subspecies and chromosomes

High levels of genetic differentiation were observed between *sharpei* and *viridis* lineages(Table 2).With the exception of one autosomal (PEPCK, Fst = 0) and one Z-linked locus (MUSK, Fst = 0.03) that were not differentiated, Fst obtained for all other 17 loci were highly significant and varied from 0.44 to 1.Accordingly, allele' networks depicted clear differentiation between *sharpei* and *viridis* lineages for most autosomal and Z-linked loci(Fig.

240 2). The mixing of nuclearalleles among geographical populations was clearly limited to the
241 contact zone (population 3, Fig. 2).Likewise, the mixing of mtDNA *sharpei* and *viridis*
242 haplotypes was also restricted to the contact zone in southern France.
243 For 60% of Z-linked loci and 44% of autosomal loci there was no sharing of alleles between
244 the two lineages (Fig. 2), and accordingly mean Fst values were very high(Fst_Z = 0.71;
245 $Fst_{autosomal}$ = 0.69). Mean net divergence divergence (Da) was higher for Z-linked loci than for
246 autosomal locibut the difference was not significant (Da_Z = 0.36%; $Da_{autosomal}$ = 0.19%;Mann-
247 Whitney test, p > 0.05).Similarly, the Z-linked loci showed a higher ratio S_{fixed}/S_{shared}(41.5%)
248 than the ratio obtained for autosomal loci (19%) (Table 2) suggesting a higher divergence of
249 Z-linked loci but the difference was not significant (Fisher exact test, p = 0.09).
250 ***Population structure***
251 Based on likelihood values, the K value selected by the DeltaK method was 2 (Fig.3).The first
252 cluster includes all *sharpei* males from Spanish populations 1 and 2 whereas the second
253 cluster comprises *viridis* males from allopatric populations 4 and 5. The overall picture is the
254 same for autosomal and Z-linked loci,with clear-cut spatial genetic differentiation between
255 *viridis* and *sharpei* lineages, and without any signs of genetic admixture except for one male
256 sampled close to the eastern limit of the contact zone (see bottom).On the other hand, within
257 the contact zone all individuals were slightly admixed at least when considering autosomal
258 loci.
259 ***Admixed birds from the secondary contact zone***
260 One male (P175) from the population 4 in Provence (*viridis* close allopatry), sampled near the
261 eastern limit of the contact zone (Fig. 1) possessed a small proportion of *sharpei* autosomal
262 genes while no introgression of *sharpei* Z-linked loci was detected (Table 3, Fig. 3). All males
263 sampled in the contact zone showed signs of slight mixed ancestry. The most eastern male of
264 the contact zone (P182) had a Z-linked *viridis* genotype (Table 3)but harbored a small

proportion of *sharpei* autosomal alleles (10%).Similarly, the juvenile male (P172) sampled on the eastern side of the contact zone possessed a high proportion of *viridis*ancestrywith15% and 5% of autosomal and Z-linked *sharpei* alleles respectively(Table 3). Both P36 and P166 males sampled acrossthe western part of the contact zone harbored a large proportion of *sharpei* ancestry, holding10% and 5% of autosomal *viridis* alleles respectively (Table 3). Overall the mean proportion of admixture was higher in autosomal loci (10%) than in Z-linked loci (3.2%) (Table 3).

Concordance between mtDNA and nuclear alleles

Nuclear alleles and mtDNA assignments were fully concordant except for one male (P172) sampled on the eastern side of the contact zone which both possessed a *sharpei* mtDNA haplotype and a large proportion of *viridis* nuclear alleles (90%). All males from populations 1 and 2 (Spain) had a *sharpei* mtDNA while all males belonging to populations 4 (Provence) and 5 (Italy) had a *viridis* mtDNA (Table 3).

Concordance between plumage and genetic lineage of admixed birds

The two males sampled from the western side of the contact zone had either a typical *sharpei*-like plumage (P166), in accordance with mtDNA and nuclear alleles assignment, or an intermediate plumage (P36) (Fig. S1). One Male (P182) sampled on the eastern side of the contact zone and one male in Provence (P175) had a typical *viridis* plumage (Fig.S1) concordant with genetic assignments.

Cline shape estimates

We used *hzar*to test whether spatial variation in allele frequencies fit a clinal model. The clinal model was statistically compared to a null model using the Akaike Information Criterium (Table S3). Results showed that the clinal model bestfit the data for 59 % of autosomal alleles and 49% of Z-linked alleles (Table S3). Not surprisingly, the null model was retained for alleles showing very low frequencies (Table S3, Table S4). Alleles that fit the

clinal model showed an abrupt change in frequencywithin the contact zone (Fig.S2a and S2b).We obtained similar values of mean center (~860 km) and mean width (~245 km) for both autosomal and Z-linked alleles. The mean cline center is located in an area between Béziers and Montpellier (Hérault).

DISCUSSION

Divergence between sharpei and viridis

STRUCTURE analyses confirmed the existence of two genetic clusters corresponding to *sharpei* and *viridis* lineages, in line with previous mitochondrial results (Pons et al. 2011).The first genetic cluster comprised males from allopatric populations 1 and 2 located in Spain (*sharpei* lineage) whereas the second cluster brought together males from populations 4 (Provence) and 5 (North-West Italy) belonging to the *viridis* lineage. Fst values obtained for most alleles also support high nuclear genetic differentiation between *sharpei* and *viridis* lineages.

Gene flow across the secondary contact zone

Our results confirm the existence of a secondary contact zone between *sharpei* and *viridis* lineages in southern France that was first described by Pons et al. (2011) based on mitochondrial genes and one Z-linked intron. The geographical mixing of *sharpei* and *viridis* alleles that are clearly differentiated at most Z-linked and autosomal loci is limited to the contact zone,i.e. to an area whose limits can be broadly delimitedin the west by the Spanish border and the Camargue when going eastward (Fig.2). More studies based on larger samples are needed to refine the geographical limits of the contact zone along the Mediterranean coast and to investigate gene flow pattern along the Pyrenean chain.

The four males sampled across the Mediterranean contact zone showedlow levels of genetic admixture (Fig.3) suggestingmoderate or low interspecific introgression, and probably well advanced speciation process. As expected from their geographical locations, the two males

sampled in the western part of the contact zone possessed a high proportion of *sharpei* alleles, whereas their eastern counterparts had mostly *viridis* alleles. Additional studies based on more individuals are required to further investigate spatial variation in gene flow across the contact zone.

We did not detect any evidence of introgression in close allopatric populations 2 (Catalonia) and 4 (Provence) except for one slightly introgressed *viridi s*male (P175). This male sampled near the contact zone in the Camargue (where only *viridis*-like green woodpeckers are currently observed by local ornithologists) possesses a small proportion of autosomal *sharpei* alleles. We thus concluded that gene flow across the contact zone isseverely restricted. This conclusion is also supported by our cline shape analysis that reveals steep clinal variations for most alleles across the *sharpei*-*viridis* contact zone. Steep clines indicate a strong reduction in gene flow that is not expected under a neutral diffusion model (Endler 1977).

A tension zone?

We tentatively argue that this secondary contact zone may function as a tension zone, resulting from a balance between dispersal of parental genotypes and selection against hybrids (Barton and Hewitt 1985). When gene flow is reduced by physical barriers or environmental factors, tension zones associated with these barriers often move and stabilizein regions of low density and dispersal. Such regions may thus act as "demographic sinks" in which immigration rates from parental populations toward the contact zonesare much higher than emigration rates from contact zones (Barton and Hewitt 1985, Hewitt 1988; Rosser et al. 2014). Several case studies provide some evidence for the long term stability of hybrid zones trapped in low density sinks(Hewitt 1988; Barrowcloughet al. 2005). In addition, gene flow across a contact zone may also be impeded by a reduction in population density due to selection and lower fitness of hybrids. In such cases,a contact zone forms a "hybrid sink" *sensu*Barton and Hewitt (1985). If demographic and hybrid sinks both operate in a given

contact zone, gene flow is strongly reduced or even completely impeded and thus clines obtained in such situations arevery steep and narrow. To date, it is not possible to know whetherslight admixture of birds sampled in the contact zone and steep clines obtained here for most alleles aredue to either “hybrid sink”or “demographic sink” effects,and how these two components might interact. However, data from the common birds monitoringprogram in France (http://vigienature.mnhn.fr/page/pic-vert) and our own unpublished observations suggest that Green woodpeckerpopulations are at very low densities in the contact zonearea (Fig. S3). This is most probably because dominant Mediterranean habitats found across the contact zone (pine forests, vineyards, dry scrublands) are not suitable,the breeding distribution o f *P. viridi s*being predominantly restricted to habitats with deciduous trees (pers. obs.; Winkler and Christie 2019).Low population density and lownatal dispersal of the Eurasian Green woodpecker (less than 10 km, see Paradis et al. 1998)bring some support to the “demographic sink” hypothesis to explain the location of the studied secondary contact zone and its probable temporal stability; green woodpeckers with intermediate plumage have been observed in this region since the beginning of the twentieth century (Jouard 1928). Interestingly, Pokrant et al.(2016) found for the Grass snake (*Natrix natrix*), a narrow secondary contact zone between Iberian and European lineages in the region of Béziers, that is geographically fully concordant with the one we describehere for the Green woodpecker (Fig. 6, Pokrant et al. 2016). In line with what is observed for the Green woodpecker, Grass snakes are very rare across the contact zone and gene flow is strongly reduced (Pokrant et al. 2016).

Further studies are needed to refine our understanding ofthe relativerole of selection and migration in reducing gene flow across the secondary *sharpei-viridis* contact zone. Nonetheless, information available to date impliesthat demographic constraints on migrationprobably play a major role in the geographical location of this contact zone.

Secondary contact zones and speciation

Most secondary contact zones between recently diverged avian taxa result from Pleistocene climatic oscillations that connected lineages having diverged in allopatric glacial refugia during cold periods. When genetically divergent lineages come into contact they may interbreed and form hybrid zones that are natural arenas for speciation research. Depending on level of gene flow, hybrid zones may be ephemeral or maintained by a balance between selection against hybrids and dispersal of parental lineages (Endler 1977). Hybrid zones are thus very useful to measure gene flow between lineages and assess whether lineages have distinct evolutionary trajectories due to reproductive isolation. Numerous avian hybrid zones have been described in western Palaearctic as well as in other biogeographical regions highlighting different speciation stages between taxa (see Price 2008 for a review). The Pyrenees are a biogeographically important region forming a suture zone where many lineages of multiple taxa endemic to the Iberian Peninsula meet their Western European counterparts (Pöschel et al. 2018).For instance, there is a genetic break in the Pyrenees between the Iberian clade of the Savi's warbler (*Locustella lucinioides*) and the Balkans clade which is largely widespread in Europe, but also considerable introgression that denotes very weak reproductive isolation (Neto et al. 2012). In the same way, there is high neutral gene flow between the carrion crow (*Corvus corone*) and the hooded crow (*Corvus cornix*) across the hybrid zone they form in Western Europe. The carrion and hooded crows maintain phenotypic divergence despite high gene flow thanks to differential expression of a small number of differentiated genes involved in plumage differences (Poelstra et al. 2014). A more advanced speciation process has been described for the Pied Flycatcher (*Ficedula hypoleuca*) and Collared flycatcher (*F. albicollis*) which expanded north and eastwards after the last glaciation and eventually came into secondary contact in central and eastern Europe. They are characterized by having efficient intrinsic post-zygotic reproductive barriers (female hybrid

390 sterility) that strongly reduce interspecific gene flow (Sæther and Sætre 2010). Hermansen et
391 al. (2011) also highlighted strong reduction in gene flow between the Italian sparrow (*Passer*
392 *italiae*) with respect to the house sparrow (*Passer domesticus*) and the Spanish sparrow
393 (*Passer hispanoliensis*) at two contact zones located in the Alps and the Gargano peninsula
394 (south-east Italy) respectively.

395 ***Patterns of introgression of Z-linked and autosomal loci***

396 The present study support slightly elevated divergence on the Z chromosome relative to
397 autosomes.Da and the ratio of fixed differences to shared polymorphisms were higher for Z-
398 linked loci. However, differences were not statistically significant probably because of the
399 low number of individuals used in the genetic analyses.Likewise, the level of admixture in the
400 contact zone was higher in autosomal loci than in Z-linked loci. We did not find any
401 differences in cline mean center and width between the two classes of markers but the low
402 sample size used to build clines prevent us to make meaningful comparisons.

403 Several avian studies on secondary contact zones and hybridization between closely related
404 species found reduced spatial introgression and higher differentiation of Z-linked loci(Sætre et
405 al. 2003;Carling and Brumfield 2008;Storchova et al. 2009; Ellegren et al. 2012;Trier et al.
406 2014; Dahmiet al. 2016; Toews et al. 2016). In such cases, differences in introgression
407 patterns and levels of genetic variation betweenZ-linked and autosomal loci cannot
408 beuniquely explained by neutral processes due to smaller effective population size of the Z
409 chromosome (Taylor et al. 2014; Manthey et al. 2015). Selection in the presence of gene flow
410 is often invokedunderlying the important role of sex chromosomes in speciation (Sæter et al.
411 2003; Dhami et al. 2016).

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Systematic issues

Our results support high genetic divergence between *sharpei* and *viridis* lineages at nuclear loci, confirming the genetic distinctiveness found by Pons et al. (2011) using mitochondrial markers and one Z-linked nuclear marker. The spatial variation of allele frequencies shows that genetic admixture is limited to a narrow geographical region located in southern France between the Spanish border and the Rhone delta. Reduced gene flow across the contact zone and low levels of admixture of birds sampled in the contact zone suggest that isolating barriers are efficient at limiting interspecific introgression. Although data concerning reproductive isolating mechanisms is currently lacking, our resultswhich need to be confirmed using a larger sample size lendsupport to the treatment of Iberian and European green woodpecker lineages as two distinct biological species *sensu* Johnson et al. (1999); interspecific gene flow is weak and spatially restricted to an apparentlystablecontact zone that is narrow relative to the breeding range of each lineage.

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LEGENDS OF FIGURES

Fig. 1 Geographic position of sampled individuals (n = 20 males) and sampled populations (n = 5). The studied contact zone between black lines (pop 3) was delimited based onthe overlap of mitochondrial lineages (Pons et al.2011), and occurrence ofindividuals with intermediate plumage (Olioso and Pons 2011). The contact zone is located in Languedoc-Roussillon along

586 the Mediterranean coast. Lowercase letters denote admixed birds: a = P36; b = P166; c =
587 P182; d = P172: e = P175
588
589 **Fig. 2** Statistical parsimony network for each nuclear locus and cytochrome b haplotypes.
590 White = allopatric populations 1 and 2 (*viridis* lineage); black = allopatric populations 4 and 5
591 (*sharpei* lineage); grey = population 3 (contact zone). The mixing of *sharpei* and *viridis*
592 nuclear alleles as well as cytochrome b haplotypes is limited to the contact zone
593
594 **Fig.3** Plots depicting the genetic structure using 16 autosomal and 18 Z-linked loci across
595 allopatric *sharpei* and *viridis* populations and across the secondary contact zone. Population
596 assignments were performed using the program STRUCTURE. Each vertical bar represents
597 one individual and shows its inferred cluster membership. Grey: *sharpei* cluster (pop 1 and
598 pop2); black: *viridis* cluster (pop4 and pop5). The model best fitting our data was K = 2.
599 Individuals from the contact zone showed low mixed ancestry.
600
601
602
603
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608 SUPPLEMENTAL MATERIAL
609 **Fig. S1**Phenotypes of the three adult males includes in the study
610 **Fig. S2a** Maximum-likelihood clines obtained with HZAR for 16 autosomal alleles (8 loci)
611 fitting the cline model
612 **Fig. S2b** Maximum-likelihood clines obtained with HZAR for 18 Z-linked alleles (7 loci)
613 fitting the cline model

Fig. S3 Relative abundance estimates of *Picus viridis* obtained from the "monitoring common birds in France" program managed by the "Centre de Recherches par le Baguage des Populations d'Oiseaux", Muséum national d'Histoire naturelle

Table S1 Information on populations, individuals and tissues

Table S2 Information on loci and primers

Table S3 Cline model parameters and values of AICc (cline model vs null model) for each Z-linked and autosomal allele

Table S4 Allelic frequencies for each Z-linked and autosomal locus

TABLES

Table 1 Positions of studied loci along the Z chromosome and polymorphism statistics obtained for 9 autosomal loci and 10 Z-linked loci.Chr[(a)] based on the *Gallus gallus* genome. Scaffold[(b)] based on the *Picoides pubescens* N307 genome assembly (GCA_000699005.1, last checked 28 august 2014). All loci but MIP (98% and 83 % similarity respectively) had only one hit according to a BLAST search. N_{bp}= number of base pairs; N_{chr} =number of chromosomes; π_{sh},π_{vi} = nucleotide diversity for *sharpei* and *viridis*; Θ_{sh},Θ_{vi} =Watterson's theta

Position	Locus	Chr[(a)]	Scaffold[(b)]		N_{bp}	N_{chr}	π_{sh}	π_{vi}	Θ_{sh}	Θ_{vi}	
Z chromosomes											
3.82	PI3kb	Z	1468	513	40	0	0.00049	0	0.00117		
7.07	KIF24	Z	292	233	40	0	0	0	0		
10.78	NIPLB	Z	46	797	40	0.00066	0.00029	0.00038	0.00038		
23.68	MIP	Z	535 and 1700	849	40	0.00059	0.00015	0.00035	0		
26.2	BRM	Z	535	329	40	0.00491	0	0.00366	0		
38.13	TLE4	Z	934	740	40	0	0	0	0		
66.51	MUSK	Z	655	533	40	0	0.00091	0	0.0017		
45.84	APC	Z	1032	911	40	0.00014	0	0.00033	0		
66.80	PLAA	Z	278	638	40	0.00056	0.00172	0.00094	0.00236		
70.81	ACO-1	Z	860	1019	40	0	0.00088	0	0.00089		
				total		average					
				6562bp			0.00069	0.00044	0.00057	0.00065	
Autosomal chromosomes											
	MB	1	25	654	40	0	0.00126	0	0.00184		
	GAPDH	1	524	403	40	0	0	0	0		
	VIM	2	1205	501	40	0.0008	0.00072	0.0006	0.00120		
	CCDC132	2	420	770	40	0	0	0	0		
	TGF2	3	134	563	40	0.00071	0	0.00054	0		
	FGB5	4	20	578	40	0.00112	0	0.0104	0		
	13336	7	1058	1700	38	0.00027	0.00025	0.00035	0.00055		
	TPM	10	560	497	40	0.00025	0	0.00061	0		
	PEPCK	20	466	686	40	0	0.00018	0	0.00044		
				total		average					
				6352bp			0.00035	0.00027	0.00140.00044		

Table 2 Genetic differentiation (Fst) and numbers of shared and fixed polymorphisms between *sharpei*(populations 1 and 2) and *viridis*lineages (populations 4 and 5). $S_{sh,}S_{vi}$ = number of shared polymorphisms for *sharpei* and *viridis* respectively. S_{fixed}= number of fixed polymorphisms. Fst = level of differentiation, Da = net nucleotide divergence. There was no statistical difference either in Fst or in Da between *sharpei* and *viridis* (Mann-Whitney test, p>0.05)

Locus	S_{sh}	S_{vi}	S_{Fixed}	Fst_{vi-sh}	Da_{vi-sh}
Z linked Chromosomes					
PI3kb	0	2	3	0.96	0.00585
NIPLB	1	1	0	0.45	0.00039
MIP	1	1	1	0.78	0.00133
BRM	4	0	3	0.83	0.01236
TLE4	0	0	2	1.0	0.00270
MUSK	0	3	0	0.03	0.00002
APC	1	0	3	0.98	0.00329
PLAA	2	5	0	0.44	0.00089
ACO-1	0	3	5	0.92	0.00558
Total	9	15	17	Mean Fst=0.71	0.00360
Autosomal chromosomes					
MB	0	4	0	0.85	0.00348
GAPDH	0	0	1	1.0	0.00248
VIM	1	2	0	0.49	0.00074
CCDC13	0	0	1	1.0	0.00130
TGF2	1	0	0	0.73	0.00098
FGB5	2	0	0	0.35	0.00030
13336	2	3	0	0.85	0.00152
TPM	1	0	2	0.97	0.00402
PEPCK	0	1	0	0 0	
Total	7	10	4	Mean Fst=0.69	0.00165

Table 3 Membership probability assignments (STRUCTURE) to *sharpei* and *viridis* cluster of admixed males sampled across the secondary contact zone and near its eastern border and MtDNA haplogroups. (a), (b), (c), (d), (e) refer to the location of individuals reported on the Figure 1

Individuals	populations	mtDNA		nuclear alleles	
		haplogroup	membership assignment	Membership coefficients	
				Z-loci	autosomal loci
(a) P36	pop3	*sharpei*	*sharpei*	*sharpei* (99%)	*sharpei* (90%)
(b) P166	pop3	*sharpei*	*sharpei*	*sharpei* (90%)	*sharpei* (95 %)
(c) P172	pop3	*sharpei*	*viridis*	*viridis*(95%)	*viridis* (85%)
(d) P182	pop3	*viridis*	*viridis*	*viridis*(100%)	*viridis* (90%)
(e) P175	pop4	*viridis*	*viridis*	*viridis*(100%)	*viridis* (90%)
				mean 96.8%	90%

Phenotypes of the three adult males from the contact zone include in the study. The fourth male was sampled as a fledgling. We calculated a plumage index (PI) based on several plumage characters (Olioso and Pons 2011, unpublished results) that varies from 1 (all plumage characters are *sharpei-type*) to 3 (all plumage characters are *viridis-type*).

Male P36, Villesèquelande, Aude, PI=2.20. This male had an intermediate plumage and mixed ancestry with a small proportion of *viridis* genes .

Male P166, Vias, central Hérault, PI=1.22. This male had a *sharpei*-type plumage and mixed ancestry with a small proportion of *viridis* genes.

Male P182, Mas de Londres, eastern Hérault, PI=2.90. This male had a *viridis*-type plumage and mixed ancestry with a small proportion of *sharpei* genes.

Male P175, Mas de la Cure, Camargue, Bouche du Rhône, PI=3. This male from population 4 (close *viridis* allopatry) with a typical *viridis* phenotype harboured a very small proportion of autosomal genes introgressed from *sharpei*.

Figure 3A: Maximum-likelihood clines obtained with HZAR for 16 autosomal alleles (8 loci) fitting the cline model.

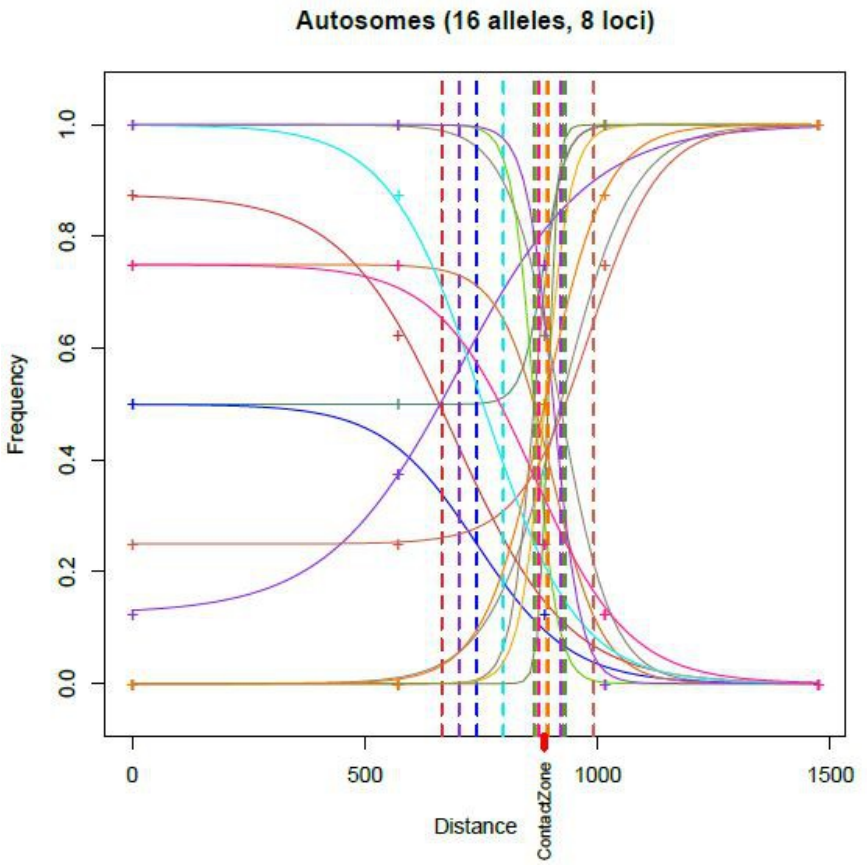


Figure 3 B: Maximum-likelihood clines obtained with HZAR for 18 Z-linked alleles (7 loci) fitting the cline model.

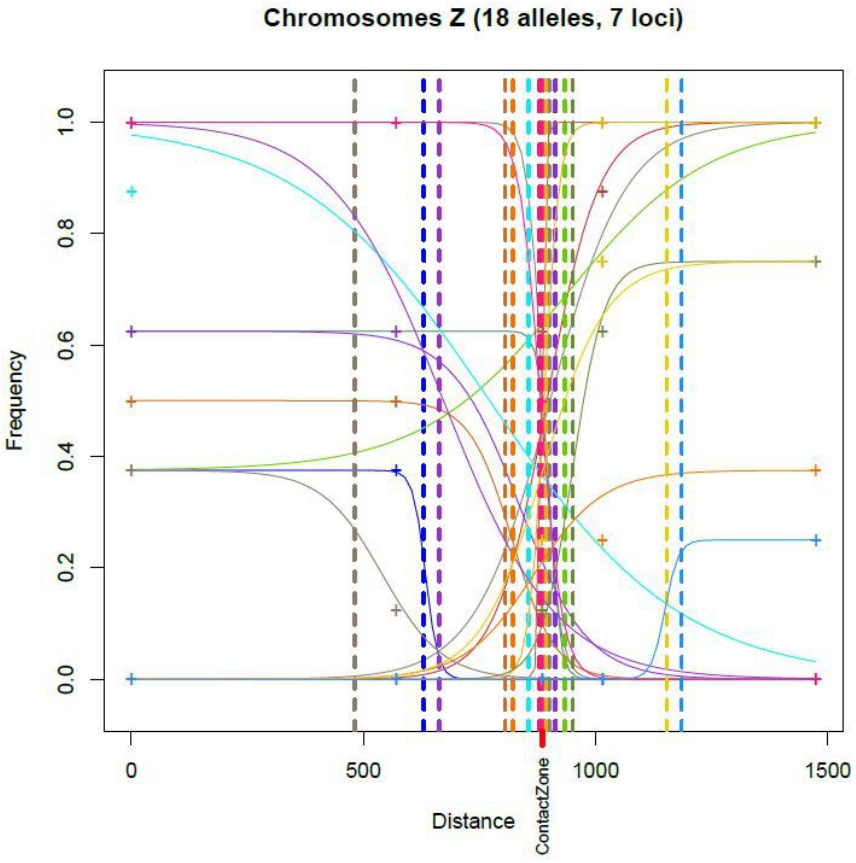
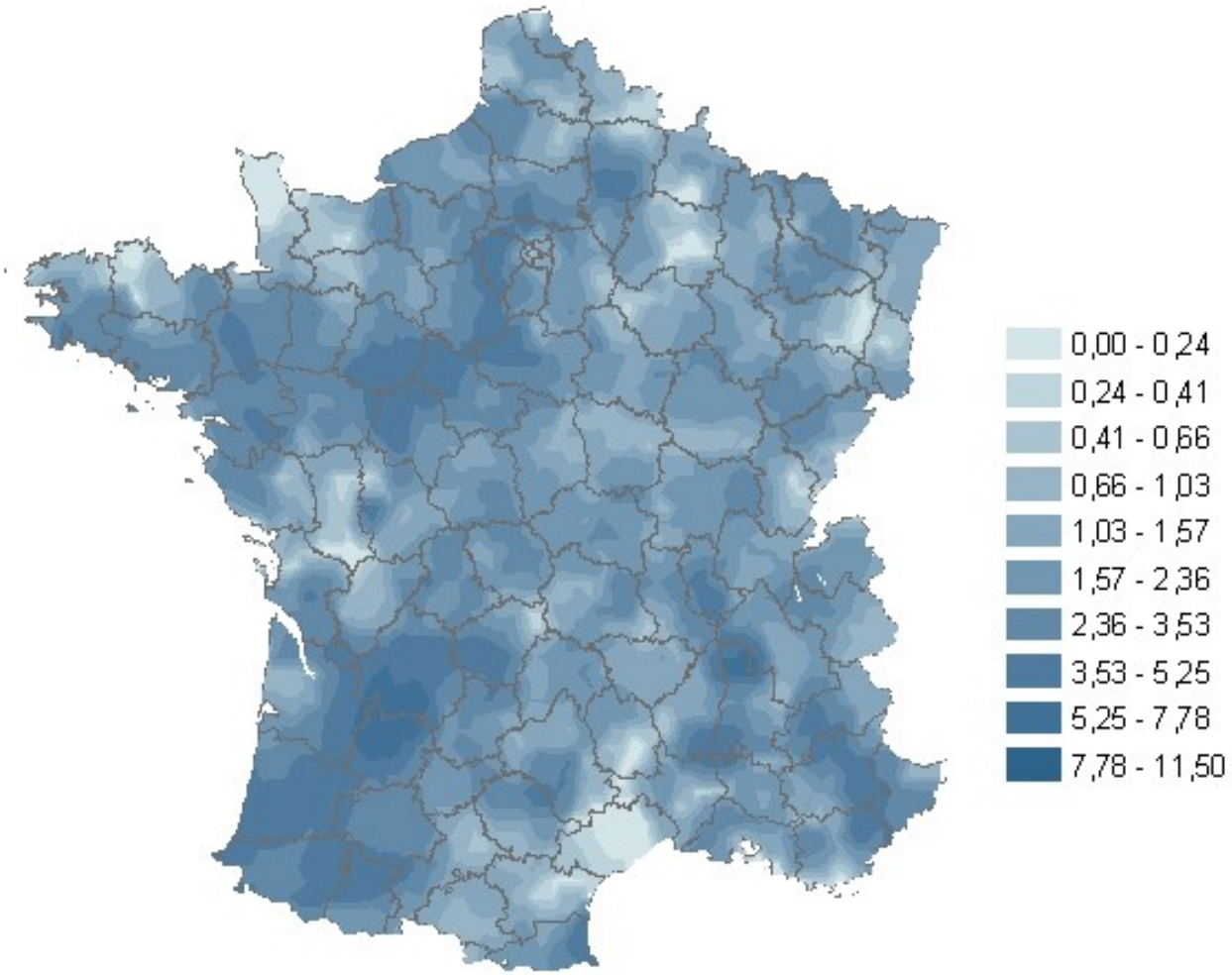


Figure S1: Relative abundance estimates of *Picus viridis* obtained from the "monitoring common birds in France" program managed by the "Centre de Recherches par le Baguage des Populations d'Oiseaux », Muséum national d'Histoire naturelle, (http://vigienature.mnhn.fr/page/pic-vert). The contact zone is centered in a region of low relative abundance between Béziers and montpellier.



Populations	Regions	individuals	Latitude	Longitude
POP1	Central Spain	P31	42.344534	-3.7129
POP1	Central Spain	P106	40.5935	-4.1475
POP1	Central Spain	P109	40.5795	-3.929
POP1	Central Spain	P110	40.3886	-4.2499
POP2	Catalonia	P62	41.6637	0.5545
POP2	Catalonia	P63	41.6104	2.2867
POP2	Catalonia	P214	41.6222	0.6478
POP2	Catalonia	P216	42.0117	2.8101
POP3	Hérault, Languedoc-Roussillon	P166	43.2912	3.3985
POP3	Gard, Languedoc-Roussillon	P172	43.5758	4.1242
POP3	Aude, Languedoc-Roussillon	P36	43.252	2.2404
POP3	Hérault, Languedoc-Roussillon	P182	43.7936	3.7988
POP4	Provence	P17	45.0251	5.0512
POP4	Provence	P58	43.621	5.0785
POP4	Provence	P92	44.3837	4.7559
POP4	Provence	P175	43.5638	4.4142
POP5	North-East Italy	P121	44.9064	7.7287
POP5	North-East Italy	P125	44.2742	9.4258
POP5	North-East Italy	P126	44.3412	9.2086
POP5	North-East Italy	P127	44.4748	9.0087

MCSN = Museo Civico di Storia Naturale di Carmagnola, Carmagnola
MSNG = Museo di Storia Naturale di Genova, Genova
MNCN = Museo Nacional de Ciencas Naturales, Madrid
MCN = Museu de Ciences Naturals, Barcelona

tissue origin	Tissue number
MNCN	MNHN-P31
MNCN	MNCN-3626
MNCN	MNCN-9661
MNCN	MNCN-3557
MCN	MCN-981372
MCN	MCN-2000-1066
MCN	MCN-2005-0908
MCN	MCN-2006-0419
Field	MNHN-P166
Field	MNHN-P172
Field	MNHN-P36
Field	MNHN-P182
Field	MNHN-P17
Field	MNHN-P58
Field	MNHN-P92
Field	MNHN-P175
MCSN	MCC-2643
MSNG	MSNG-627
MSNG	MSNG-629
MSNG	MSNG-631

Locus	Gene description	Chromosome
PI3kb		Z
KIF24	Kinesin Family Member 24	Z
NIPLB	Nipped-B homolog	Z
MIP		Z
BRM		Z
TLE4	Transducin-like eEnhancer of split 4	Z
MUSK	Muscle, skeletal, receptor tyrosine kinase	Z
APC	Adenomatous Polyposis Coli	Z
PLAA	Phospholipase A-2-activating protein	Z
ACO-1	aconitase 1, soluble 1 intron-9	Z
MB	Myoglobin intron-2	1
GAPDH		1
VIM	Vimentin intron-8	2
CCDC132	coiled-coil domain containing 132	2
TGF2	Transforming Growth Factor beta 2 intron-5	3
FGB5	Fibrinogen beta chain, intron 5	4
13336	eukaryotic translation initiation factor 3, subunit 1	7
TPM	Tropomyosin alpha intron-6	10
PEPCK	Phosphoenol Pyruvate Carboxykinase intron-9	20

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Forward Primer: 5'-3'	Reverse Primer: 5'-3'
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GATCAGGAACACACACATAC	CTTGGAATTGCCAATGAAAG
GCAGAACACTCTCTATCCTCA	TTGTGGGTGGAACACTTGG
ATCATGGTACATATCCCACG	TCTTGGTTACGTTTGTCCT
AGCACCTTTGAACAGTGGTT	TACTTTATGGAGACGACGGA
AGAGTGATGACAACTTGGT	GCTAAGTTCTTTGGATTTGGA
CTTCCATGCACTACAATGGGA	CTCTGAACATTGTGGATCCTCAA
GTGAGTCCATACATTTCACA	CTTTCATTTGATGAAGAACACA
CCTGTATCTCCTCGGCACTT	GTTCAAACAATCAGACTCCC
CTGTGGGAATGCTGAGAGAT	CTGCAGCAAGGCACAACAGT
GCCACCAAGCACAAGATCCC	TTCAGCAAGGACCTTGATAATGAC
TCCACCTTTGATGCGGGTGC	AAGTCCACAACACGGTTGCTGTA
GACCGTGGAAACTAGAGATG	GTCATCGTGATGCTGGGAAGTTTC
TCTGGGAACAGATCTGTC	AAACTTCAGACTTACTGCC
GAAGCGTGCTCTAGATGCTG	AGGCAGCAATTATCCTGCAC
CGCCATACAGAGTATACTGTC	GCCATCCTGGCGATTCTGAA
CAGTTAGCAGACAAACTACG	GGTTCATGGCATCTATTCC
AATGGCTGCAGAGGATAA	TCCTCTTCAAGCTCAGCACA
GGAGCAGCCATGAGATCTGA	GTGCCATGCTAAGCCAGTGGG

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50, no. 3 (2009): 654-660.

Cline model parameters and values of AICc (cline model vs null model) for each allele. Selected models are highlihted in yellow.

Allele	Center	Width	CenterLow	CenterHigh	WidthLow	WidthHigh	AICc_ClineModel	AICc_NullModel
Autosomal chromosomes								
A1_1 (TGBF)	919.0889	223.9556	765.5652	1026.378	138.6084	903.5128	4.753327	21.441108
A1_2	920.3587	309.4908	775.5458	1095.074	173.0496	1045.821	4.814646	19.705220
A1_3	1241.124	1324.236	39.68369	1989.669	12.38007	1997.522	6.220534	3.884335
A2_1 (FGB)	931.0603	288.1394	409.639	1058.699	14.99286	2003.627	4.722313	9.126592
A2_2	740.2052	402.7596	102.1862	1029.907	293.3657	2006.531	5.029764	8.386475
A2_3	619.2052	648.1064	33.69199	1929.656	14.67431	2001.657	5.774163	3.884335
A2_4	1574.106	1973.414	60.51943	1995.424	4.810192	2001.135	6.423749	3.884335
A3_1 (MB)	665.3018	516.8475	374.6027	878.1707	143.542	1847.781	5.472967	15.314739
A3_2	702.0647	567.1107	319.4978	901.1328	96.6271	1896.392	5.553879	15.314739
A4_1 (PEPCK)	866.6605	56.99928	710.2213	923.1849	56.99928	665.0252	4.845057	24.605583
A4_2	863.6167	99.08844	699.3543	934.7498	66.39714	649.4056	4.849083	24.605583
A5_1(VIM)	926.042	584.3886	652.0618	1145.093	198.5318	2003.551	4.872092	11.939706
A5_2	992.5816	560.923	767.5738	1277.826	151.2236	2003.037	4.77652	11.10954
A5_3	774.3411	218.2	10.98025	1997.353	144.703	2004.262	5.805796	4.134509
A6_1(Tropo)	797.9372	310.3551	572.8774	930.0112	285.0458	1135.811	5.843882	21.441108
A6_2	750.2443	135.3972	88.43056	1991.234	30.28116	1967.722	5.792622	3.884335
A6_3	890.3109	64.07703	770.6765	953.1716	29.85833	630.7576	4.781243	24.402932
A6_4	1447.567	1735.996	54.86903	1918.833	10.38916	1987.731	6.427482	3.884335
A7_1 (13336)	919.8383	75.90972	824.3952	988.0308	24.48679	483.011	5.034487	25.249094
A7_2	926.0621	120.4137	827.628	980.0716	39.06406	422.3955	4.771803	25.249094
A8_1 (15463)	873.3807	205.1678	490.5605	1029.772	205.1678	1934.597	4.92749	11.21624
A8_2	929.3623	624.1641	72.61918	1983.786	105.6389	1990.91	5.296442	3.688065
A8_3	768.3074	321.3689	11.04352	1973.546	6.144715	1997.366	4.779275	4.042269
A8_4	894.3513	159.9038	731.0851	1010.944	122.4831	936.9008	4.814469	19.804631
A8_5	1329.43	1470.6	-4.658006	1976.312	128.2834	1973.108	6.342819	3.845444
A9_1 (GAPDH)	1474.102	386.3424	14.3012	1948.459	49.34782	1994.405	4.707184	3.884335
A9_2	1333.023	121.7224	43.25445	1966.168	52.24649	2008.73	4.705964	3.884335
Z chromosomes								
Z1_1 (PLAA)	887.2983	65.84432	783.5733	947.1201	65.84432	600.9953	4.721093	24.402932
Z1_2	900.262	255.3061	732.1032	1047.074	151.6379	1199.722	4.950712	19.703916
Z1_3	1646.665	1922.876	53.80408	1922.012	57.55326	2007.479	5.786417	3.884335
Z1_4	1561.647	1528.295	11.22061	1988.678	25.20363	2009.51	5.783170	3.884335
Z3_1 (MIP)	914.9303	95.86447	740.9267	1036.436	20.78074	1225.287	4.70615	11.98990
Z3_2	629.8994	72.70194	93.73737	916.3718	53.09672	1977.765	4.706050	8.545574
Z3_3	879.1812	203.3416	722.9714	984.6182	167.9203	967.3145	4.78089	21.33875
Z3_4	925.8438	319.3016	-9.14935	1974.422	7.444824	2006.073	5.973539	3.884335
Z4_1 (TLE)	913.1575	26.91679	300.9608	1121.664	26.91679	1967.516	5.869856	10.526605
Z4_2	934.1781	616.3051	574.4519	1465.617	195.0745	1978.755	4.944840	7.491217
Z4_3	1311.494	1711.913	74.69375	1991.691	129.5431	1960.343	7.860444	5.664151
Z5_1 (BRM)	788.6394	247.3172	20.56719	1979.328	93.37291	1999.737	4.719560	4.134509
Z5_2	481.447	658.2569	3.213405	839.5466	62.93184	1956.674	4.736991	6.918874
Z5_3	806.0657	326.1124	256.7067	963.1557	94.61485	2002.391	4.793209	9.444260
Z5_4	718.6067	571.5773	-5.289438	1864.819	16.4456	2005.837	6.048467	3.884335
Z5_5	861.3476	832.7437	47.01951	1985.377	153.691	1979.857	6.201272	4.134509
Z5_6	884.7348	15.27941	696.9767	928.6462	3.974197	642.8208	4.797517	24.605583
Z5_7	1615.124	1988.762	6.85045	1999.24	20.36203	2004.297	6.434537	3.884335
Z6_1 (MUSK)	877.8542	84.31066	744.0788	952.75	22.59483	599.1544	4.787712	24.402932
Z6_2	886.8571	79.22981	734.2873	946.2864	65.69012	631.6721	4.722719	24.402932
Z7_1 (AC01)	1039.206	851.0395	134.7147	1948.245	107.4853	2003.803	5.960296	5.364758
Z7_2	953.6033	354.3714	110.3258	1955.661	267.0036	2003.812	7.856021	5.664151
Z7_3	1323.403	543.407	31.59175	1936.47	8.67113	2009.992	4.705882	3.884335
Z8_1 (APC)	855.9311	622.0738	502.6173	1020.785	412.6355	1975.669	10.87987	21.33875
Z8_2	893.217	130.0769	749.6284	955.0315	63.51724	624.4927	4.772443	24.402932
Z9_1 (NIPBL)	663.6332	474.08	371.6156	836.85	348.821	1422.18	5.599695	18.893565
Z9_2	832.1492	521.9755	80.61068	1721.741	108.8059	2001.973	7.134843	5.364758
Z9_3	808.4398	92.48207	0.9867241	1960.977	3.001898	2007.828	5.774133	3.884335
Z9_4	806.4137	225.0104	0.05423641	1934.184	13.70488	2004.184	5.814498	4.134509
Z9_5	951.5785	243.2939	776.237	1173.36	81.73211	1283.17	4.754197	13.851446
Z9_6	789.9927	629.617	19.34888	1895.631	92.51917	2005.039	6.428432	6.325237
Z9_7	1002.603	523.5969	63.2781	1998.777	97.34961	2008.206	6.156407	3.884335
Z10_1 (PI3Kb)	881.9487	91.97031	747.54	947.5777	81.1725	563.725	4.753933	24.402932
Z10_2	822.3224	446.0327	235.3817	1556.674	13.58341	2007.312	4.918482	6.481412
Z10_3	1153.221	1577.38	676.7919	1670.74	270.2765	2009.829	9.596722	11.219585
Z10_4	1185.944	112.2032	732.416	1944.894	57.93866	1995.251	4.706066	5.664151
Z10_5	1309.782	512.6949	41.80314	1964.391	139.0911	2001.921	4.867151	3.884335

Allele	Chromosor	Center	Width	CenterLow	CenterHigh	WidthLow
A1_1	A	919.0889	223.9556	765.5652	1026.378	138.6084
A1_2	A	920.3587	309.4908	775.5458	1095.074	173.0496
A2_1	A	931.0603	288.1394	409.639	1058.699	14.99286
A2_2	A	740.2052	402.7596	102.1862	1029.907	293.3657
A3_1	A	665.3018	516.8475	374.6027	878.1707	143.542
A3_2	A	702.0647	567.1107	319.4978	901.1328	96.6271
A4_1	A	866.6605	56.99928	710.2213	923.1849	56.99928
A4_2	A	863.6167	99.08844	699.3543	934.7498	66.39714
A5_1	A	926.042	584.3886	652.0618	1145.093	198.5318
A5_2	A	992.5816	560.923	767.5738	1277.826	151.2236
A6_1	A	797.9372	310.3551	572.8774	930.0112	285.0458
A6_3	A	890.3109	64.07703	770.6765	953.1716	29.85833
A7_1	A	919.8383	75.90972	824.3952	988.0308	24.48679
A7_2	A	926.0621	120.4137	827.628	980.0716	39.06406
A8_1	A	873.3807	205.1678	490.5605	1029.772	205.1678
A8_4	A	894.3513	159.9038	731.0851	1010.944	122.4831
Z1_1	Z	887.2983	65.84432	783.5733	947.1201	65.84432
Z1_2	Z	900.262	255.3061	732.1032	1047.074	151.6379
Z3_1	Z	914.9303	95.86447	740.9267	1036.436	20.78074
Z3_2	Z	629.8994	72.70194	93.73737	916.3718	53.09672
Z3_3	Z	879.1812	203.3416	722.9714	984.6182	167.9203
Z4_1	Z	913.1575	26.91679	300.9608	1121.664	26.91679
Z4_2	Z	934.1781	616.3051	574.4519	1465.617	195.0745
Z5_2	Z	481.447	658.2569	3.213405	839.5466	62.93184
Z5_3	Z	806.0657	326.1124	256.7067	963.1557	94.61485
Z5_6	Z	884.7348	15.27941	696.9767	928.6462	3.974197
Z8_1	Z	855.9311	622.0738	502.6173	1020.785	412.6355
Z8_2	Z	893.217	130.0769	749.6284	955.0315	63.51724
Z9_1	Z	663.6332	474.08	371.6156	836.85	348.821
Z9_5	Z	951.5785	243.2939	776.237	1173.36	81.73211
Z10_1	Z	881.9487	91.97031	747.54	947.5777	81.1725
Z10_2	Z	822.3224	446.0327	235.3817	1556.674	13.58341
Z10_3	Z	1153.221	1577.38	676.7919	1670.74	270.2765
Z10_4	Z	1185.944	112.2032	732.416	1944.894	57.93866

WidthHigh	AICc_Cline	AICc_NullModel
903.5128	4.753327	21.441108
1045.821	4.814646	19.705220
2003.627	4.722313	9.126592
2006.531	5.029764	8.386475
1847.781	5.472967	15.314739
1896.392	5.553879	15.314739
665.0252	4.845057	24.605583
649.4056	4.849083	24.605583
2003.551	4.872092	11.939706
2003.037	4.77652	11.10954
1135.811	5.843882	21.441108
630.7576	4.781243	24.402932
483.011	5.034487	25.249094
422.3955	4.771803	25.249094
1934.597	4.92749	11.21624
936.9008	4.814469	19.804631
600.9953	4.721093	24.402932
1199.722	4.950712	19.703916
1225.287	4.70615	11.98990
1977.765	4.706050	8.545574
967.3145	4.78089	21.33875
1967.516	5.869856	10.526605
1978.755	4.944840	7.491217
1956.674	4.736991	6.918874
2002.391	4.793209	9.444260
642.8208	4.797517	24.605583
1975.669	10.87987	21.33875
624.4927	4.772443	24.402932
1422.18	5.599695	18.893565
1283.17	4.754197	13.851446
563.725	4.753933	24.402932
2007.312	4.918482	6.481412
2009.829	9.596722	11.219585
1995.251	4.706066	5.664151

Allele	Chromosor	Center	Width	CenterLow	CenterHigh	WidthLow
A1_1	A	919.0889	223.9556	765.5652	1026.378	138.6084
A1_2	A	920.3587	309.4908	775.5458	1095.074	173.0496
A3_1	A	665.3018	516.8475	374.6027	878.1707	143.542
A3_2	A	702.0647	567.1107	319.4978	901.1328	96.6271
A4_1	A	866.6605	56.99928	710.2213	923.1849	56.99928
A4_2	A	863.6167	99.08844	699.3543	934.7498	66.39714
A5_1	A	926.042	584.3886	652.0618	1145.093	198.5318
A6_1	A	797.9372	310.3551	572.8774	930.0112	285.0458
A6_3	A	890.3109	64.07703	770.6765	953.1716	29.85833
A7_1	A	919.8383	75.90972	824.3952	988.0308	24.48679
A7_2	A	926.0621	120.4137	827.628	980.0716	39.06406
A8_4	A	894.3513	159.9038	731.0851	1010.944	122.4831
Z1_1	Z	887.2983	65.84432	783.5733	947.1201	65.84432
Z1_2	Z	900.262	255.3061	732.1032	1047.074	151.6379
Z3_3	Z	879.1812	203.3416	722.9714	984.6182	167.9203
Z5_6	Z	884.7348	15.27941	696.9767	928.6462	3.974197
Z8_1	Z	855.9311	622.0738	502.6173	1020.785	412.6355
Z8_2	Z	893.217	130.0769	749.6284	955.0315	63.51724
Z9_1	Z	663.6332	474.08	371.6156	836.85	348.821
Z9_5	Z	951.5785	243.2939	776.237	1173.36	81.73211
Z10_1	Z	881.9487	91.97031	747.54	947.5777	81.1725

WidthHigh	AICc_ClineModel	AICc_NullModel
903.5128	4.753327	21.441108
1045.821	4.814646	19.705220
1847.781	5.472967	15.314739
1896.392	5.553879	15.314739
665.0252	4.845057	24.605583
649.4056	4.849083	24.605583
2003.551	4.872092	11.939706
1135.811	5.843882	21.441108
630.7576	4.781243	24.402932
483.011	5.034487	25.249094
422.3955	4.771803	25.249094
936.9008	4.814469	19.804631
600.9953	4.721093	24.402932
1199.722	4.950712	19.703916
967.3145	4.78089	21.33875
642.8208	4.797517	24.605583
1975.669	10.87987	21.33875
624.4927	4.772443	24.402932
1422.18	5.599695	18.893565
1283.17	4.754197	13.851446
563.725	4.753933	24.402932

Population	Transect		% missing	A1_1	A1_2
Madrid		0	0.000	1.000	0.000
Barcelone	570.808585418926		0.000	1.000	0.000
ZoneContact	885.504968523781		0.000	0.125	0.750
ValleeRhone	1015.34604260266		0.000	0.000	1.000
Italie	1474.25051022629		0.000	0.625	0.375
Overall			0.000	0.550	0.425

A1_3
0.000
0.000
0.125
0.000
0.000
0.025

Population	Transect	% missing	A2_1	A2_2	A2_3	A2_4
Madrid	0	0.000	0.500	0.500	0.000	0.000
Barcelone	570.8085854	0.000	0.500	0.375	0.125	0.000
ZoneContact	885.5049685	0.000	1.000	0.000	0.000	0.000
ValleeRhone	1015.346042	0.000	1.000	0.000	0.000	0.000
Italie	1474.250510	0.000	0.750	0.125	0.000	0.125
Overall		0.000	0.750	0.200	0.025	0.025

Population	Transect	% missing	A3_1	A3_2
Madrid	0	0.000	0.875	0.125
Barcelone	570.8085854	0.000	0.625	0.375
ZoneContact	885.5049685	0.000	0.000	1.000
ValleeRhone	1015.346042	0.000	0.000	1.000
Italie	1474.250510	0.000	0.250	0.750
Overall		0.000	0.350	0.650

Population	Transect	% missing	A4_1	A4_2
Madrid	0	0.000	1.000	0.000
Barcelone	570.808585	40.000	1.000	0.000
ZoneContact	885.504968	50.000	0.000	1.000
ValleeRhone	1015.346042	0.000	0.000	1.000
Italie	1474.250510	0.000	0.375	0.625
Overall		0.000	0.475	0.525

Population	Transect	% missing	A5_1	A5_2	A5_3
Madrid	0	0.000	0.750	0.250	0.000
Barcelone	570.8085854	0.000	0.750	0.250	0.000
ZoneContact	885.5049685	0.000	0.125	0.750	0.125
ValleeRhone	1015.346042	0.000	0.000	1.000	0.000
Italie	1474.250510	0.000	0.500	0.375	0.125
Overall		0.000	0.425	0.525	0.050

Population	Transect	% missing	A6_1	A6_2	A6_3	A6_4
Madrid	0	0.000	1.000	0.000	0.000	0.000
Barcelone	570.8085854	0.000	0.875	0.125	0.000	0.000
ZoneContact	885.5049685	0.000	0.000	0.000	1.000	0.000
ValleeRhone	1015.346042	0.000	0.000	0.000	1.000	0.000
Italie	1474.250510	0.000	0.375	0.000	0.500	0.125
Overall		0.000	0.450	0.025	0.500	0.025

Population	Transect	% missing	A7_1	A7_2
Madrid	0	0.000	1.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000
ZoneContact	885.5049685	0.000	0.000	1.000
ValleeRhone	1015.346042	0.000	0.000	1.000
Italie	1474.250510	0.000	0.750	0.250
Overall		0.000	0.550	0.450

Population	Transect	% missing	A8_1	A8_2	A8_3	A8_4
Madrid	0	0.000	0.750	0.125	0.125	0.000
Barcelone	570.8085854	0.000	0.750	0.125	0.125	0.000
ZoneContact	885.5049685	0.000	0.125	0.000	0.000	0.875
ValleeRhone	1015.346042	0.250	0.000	0.000	0.000	1.000
Italie	1474.250510	0.000	0.250	0.125	0.000	0.500
Overall		0.050	0.395	0.079	0.053	0.447

A8_5
0.000
0.000
0.000
0.000
0.125
0.026

Population	Transect	% missing	A9_1	A9_2
Madrid	0	0.000	1.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000
ZoneContact	885.5049685	0.000	1.000	0.000
ValleeRhone	1015.346042	0.000	0.875	0.125
Italie	1474.250510	0.000	1.000	0.000
Overall		0.000	0.975	0.025

Population	Transect	% missing	Z1_1	Z1_2	Z2_3	Z2_4
Madrid	0	0.000	1.000	0.000	0.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000	0.000	0.000
ZoneContact	885.5049685	0.000	0.000	0.750	0.125	0.125
ValleeRhone	1015.346042	0.000	0.000	1.000	0.000	0.000
Italie	1474.250510	0.000	0.500	0.500	0.000	0.000
Overall		0.000	0.500	0.450	0.025	0.025

Population	Transect	% missing	Z2_1
Madrid	0	0.000	1.000
Barcelone	570.8085854	0.000	1.000
ZoneContact	885.5049685	0.000	1.000
ValleeRhone	1015.346042	0.000	1.000
Italie	1474.250510	0.000	1.000
Overall		0.000	1.000

Population	Transect	% missing	Z3_1	Z3_2	Z3_3	Z3_4
Madrid	0	0.000	0.625	0.375	0.000	0.000
Barcelone	570.8085854	0.000	0.625	0.375	0.000	0.000
ZoneContact	885.5049685	0.000	0.000	0.000	0.875	0.125
ValleeRhone	1015.346042	0.000	0.000	0.000	1.000	0.000
Italie	1474.250510	0.000	0.500	0.000	0.500	0.000
Overall		0.000	0.350	0.150	0.475	0.025

Population	Transect	% missing	Z4_1	Z4_2	Z4_3
Madrid	0	0.000	0.625	0.375	0.000
Barcelone	570.8085854	0.000	0.500	0.500	0.000
ZoneContact	885.5049685	0.000	0.000	0.750	0.250
ValleeRhone	1015.346042	0.000	0.000	1.000	0.000
Italie	1474.250510	0.000	0.375	0.625	0.000
Overall		0.000	0.300	0.650	0.050

Population	Transect	% missing	Z5_1	Z5_2	Z5_3	Z5_4
Madrid	0	0.000	0.125	0.375	0.500	0.000
Barcelone	570.8085854	0.000	0.125	0.125	0.500	0.125
ZoneContact	885.5049685	0.000	0.000	0.000	0.000	0.000
ValleeRhone	1015.346042	0.000	0.000	0.000	0.000	0.000
Italie	1474.250510	0.000	0.000	0.000	0.125	0.000
Overall		0.000	0.050	0.100	0.225	0.025

Z5_5	Z5_6	Z5_7
0.000	0.000	0.000
0.125	0.000	0.000
0.000	1.000	0.000
0.000	1.000	0.000
0.125	0.625	0.125
0.050	0.525	0.025

Population	Transect	% missing	Z6_1	Z6_2
Madrid	0	0.000	1.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000
ZoneContact	885.5049685	0.000	0.000	1.000
ValleeRhone	1015.346042	0.000	0.000	1.000
Italie	1474.250510	0.000	0.500	0.500
Overall		0.000	0.500	0.500

Population	Transect	% missing	Z7_1	Z7_2	Z7_3
Madrid	0	0.000	1.000	0.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000	0.000
ZoneContact	885.5049685	0.000	0.750	0.250	0.000
ValleeRhone	1015.346042	0.000	0.875	0.000	0.125
Italie	1474.250510	0.000	1.000	0.000	0.000
Overall		0.000	0.925	0.050	0.025

Population	Transect	% missing	Z8_1	Z8_2	Z8_3
Madrid	0	0.000	0.875	0.000	0.125
Barcelone	570.8085854	0.000	1.000	0.000	0.000
ZoneContact	885.5049685	0.000	0.000	1.000	0.000
ValleeRhone	1015.346042	0.000	0.000	1.000	0.000
Italie	1474.250510	0.000	0.500	0.500	0.000
Overall		0.000	0.475	0.500	0.025

Population	Transect	% missing	Z9_1	Z9_2	Z9_3	Z9_4
Madrid	0	0.000	1.000	0.000	0.000	0.000
Barcelone	570.8085854	0.000	0.625	0.250	0.125	0.000
ZoneContact	885.5049685	0.000	0.000	0.000	0.000	0.125
ValleeRhone	1015.346042	0.000	0.000	0.000	0.000	0.000
Italie	1474.250510	0.000	0.250	0.125	0.000	0.125
Overall		0.000	0.375	0.075	0.025	0.050

Z9_5	Z9_6	Z9_7
0.000	0.000	0.000
0.000	0.000	0.000
0.625	0.125	0.125
0.750	0.250	0.000
0.125	0.375	0.000
0.300	0.150	0.025

Population	Transect	% missing	Z10_1	Z10_2	Z10_3	Z10_4
Madrid	0	0.000	1.000	0.000	0.000	0.000
Barcelone	570.8085854	0.000	1.000	0.000	0.000	0.000
ZoneContact	885.5049685	0.000	0.000	0.250	0.750	0.000
ValleeRhone	1015.346042	0.000	0.000	0.375	0.250	0.250
Italie	1474.250510	0.000	0.500	0.250	0.250	0.000
Overall		0.000	0.500	0.175	0.250	0.050

Z10_5
0.000
0.000
0.000
0.125
0.000
0.025