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Hybridisation and climate change: brown argus butterflies in Britain (*Polyommatus* subgenus *Aricia*)

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Abstract. 1. Distribution changes brought about by climate change are likely to alter levels of hybridisation between related taxa, and may threaten some species.

2. Nuclear (*Tpi*) and mitochondrial (*cytB*) DNA sequence data give evidence for introgression between two related *Polyommatus* (subgenus *Aricia*) butterfly species in a 150–200 km wide overlap zone in northern England and North Wales. A history of hybridisation is evident from the mixture of genotypes present within this region: some populations contain southern-origin (*Polyommatus agestis*) mtDNA and northern-origin (*Polyommatus artaxerxes*) *Tpi* alleles, and many populations contain mixtures of *Tpi* alleles.

3. The timing of the original hybridisation is unknown, but could be immediately post-glacial or much more recent in origin.

4. Both species are now beginning to shift northwards, associated with recent climatic warming.

5. It is thus expected that anthropogenic climate change will unleash a new spate of hybridisation, potentially threatening the long-term survival of the northern species in Britain.

Key words. Adaptation, climate warming, conservation, distributions, extinction, hybridisation, Lepidoptera, Lycaenidae, voltinism.

Introduction

Many species of animal, including butterflies, periodically hybridise with other recognised species (Arnold, 1997; Mallet, 2005, 2008; Mallet *et al*., 2007; Descimon & Mallet, 2009), and hybrid zones between related species or geographic subspecies are commonly observed (Barton & Hewitt, 1989; Jiggins & Mallet, 2000). Hybridisation is recognised as a common threat to biodiversity, particularly when species are accidentally or deliberately moved from one part of the world, or when anthropogenic habitat changes permit one species to colonise new regions (Allendorf *et al*., 2001). When previously geographically-separated taxa meet, hybridisation may result in the loss of one of the former taxa through genetic swamping, or a new taxon may form containing elements of the former genes of each species (Rhymer & Simberloff, 1996; Mallet, 2007).

Climate change is today resulting in widespread changes to the distributions of species, including insects (e.g., Parmesan *et al*., 1999; Warren *et al*., 2001; Parmesan & Yohe, 2003; Hickling *et al*., 2006; Chen *et al*., 2009). Climate change may bring the distributions of formerly separated species into geographic contact, or it may isolate populations of a single species and lead to eventual divergence. Current geographic patterns of racial differentiation within species, and the locations of hybrid zones have been argued to reflect the effects of past glacial and interglacial climates on the historical distributions of species (e.g., Hewitt, 1999). The unprecedented current anthropogenic climate change is likely, therefore, to generate major shifts in location and level of introgression among species.

Here, we report on the current geographic distribution and hybridisation in Britain of two closely-related lineages within *Polyommatus* (subgenus *Aricia*) butterflies today considered separate species on the basis of biological and genetic differences (Thomas & Lewington, 1991; Tolman & Lewington, 1997; Aagaard *et al*., 2002). In Britain, the brown argus, *Polyommatus* (*Aricia*) *agestis* is restricted to southern areas, where it has been regarded as bivoltine (two adult flight periods and generations

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per year). In contrast, the univoltine (with a single flight period) northern brown argus, *Polyommatus* (*Aricia*) *artaxerxes*, is considered restricted to northern England and Scotland. Molecular data from mtDNA and allozymes generally conformed with morphological information and voltinism, and clearly demonstrated two genetic lineages, both widely distributed across Europe. In Western Europe, the northern brown argus, *P. artaxerxes*, occurs in northern Britain and most of Scandinavia [Norway, Sweden, Jylland (Jutland) in Denmark], while the brown argus, *P. agestis*, occurs widely in southern and central Europe, as well as in southern Britain and southern Sweden, and also in Jylland, Denmark, where it is reported to overlap with *P. artaxerxes* (Aagaard *et al*., 2002). Although *P. artaxerxes* populations in the type locality, Scotland, have a distinct colour pattern (well-developed forewing medial white spot), they group genetically with northern English and mainland European *P. artaxerxes* populations (Aagaard *et al*., 2002) which often lack this spot. *Polyommatus agestis* in England is currently expanding northwards very rapidly (Asher *et al*., 2001; Thomas *et al*., 2001; Fox *et al*., 2006). Meanwhile, *P. artaxerxes*, is starting to retreat northwards (Franco *et al*., 2006).

Colour pattern and host plant usage also vary between *P. agestis* and *P. artaxerxes* (e.g., Emmet & Heath, 1990; Thomas & Lewington, 1991; Dennis, 1992; Smyllie, 1996, 2010). There has been some question about the species assignments of some populations in northern England (Smyllie, 1996, 2010). All univoltine populations were assigned to *P. artaxerxes* in the first national butterfly distribution atlas (Heath *et al*., 1984), and the southernmost of these univoltine populations were referred to *P. agestis* in the second atlas (Asher *et al*., 2001). Our results here show that the distinction is somewhat arbitrary because nuclear genes within the northern England and North Wales populations originate from both species. Nonetheless, analyses of mtDNA show that the two forms are characterised by separate mitochondrial lineages, as well as by other genetic differences (below) that have been considered sufficient to support their status as separate species (Aagaard *et al*., 2002).

Here we employ DNA sequence data from a nuclear gene, *Triose phosphate isomerase* (*Tpi*), to investigate potential introgression between these two taxa across a broad band in northern England. We discuss the species delimitation and conservation implications of these discoveries.

Material and methods

Samples were collected from the British populations shown in Table 1. Voltinism of these populations was deduced from direct observation as well as Butterfly Monitoring Scheme transects available from each region.

Mitochondrial DNA data was taken from *cytB* sequences in a previous study (Aagaard *et al*., 2002), supplemented with new material amplified and sequenced according to those authors' protocols. Preliminary allozyme evidence suggested differentiation and potentially mixed populations in northern England for the enzyme locus *Triose phosphate isomerase* (*Tpi*) (Aagaard *et al*., 2002). We followed this lead and developed PCR primers for amplifying a diagnostic region of this locus in *Polyommatus* (*Aricia*) (hereafter '*Aricia*'). First, we used existing primers developed initially for *Tpi* in *Heliothis virescens* and *Heliconius* (Beltrán *et al*., 2002), in order to obtain DNA sequence information from *Aricia*. The resulting sequences were compared and aligned to complete *Tpi* gene sequences in GENBANK, consisting of human and *Drosophila* sequences, as well as those of two Lepidoptera, *Heliconius* and *Heliothis*. *Tpi* is usually sex-linked in Lepidoptera, and this proved to be the case also in *Aricia*. Therefore, to simplify sequencing and avoid ambiguous base calls or indel effects due to mixed haplotypes of different lengths in heterozygotes, we sequenced *Tpi* from hemizygous females only (in Lepidoptera, females are the heterogametic sex, giving effective haploidy for sex-linked loci). The first five exons of *Tpi* and intervening introns were sequenced by 'walking' along the gene from the region amplified by the generalized Lepidoptera primers. A region between exons 1 and 3, consisting of about 700–1100 bp was found to be diagnostic between the two species, and PCR primers were designed from the *Aricia* sequences: Daz (forward, in exon 1) ATGTCATTGCAGGTG GTAGTGGGAGT and EL (reverse, in exon 3) TCTGAACG-CAGAACAATCTTC. We have made available our new sequence information from these populations via Genbank accession nos. HQ117948-HQ117987 for *cytB* and HQ117988-HQ118099 for *Tpi*.

Results

As already known (Aagaard *et al*., 2002), *P. agestis* and *P. artaxerxes* differ at a number of fixed sites in the mitochondrial locus *cytB*, giving on average a 4.0% difference between the species, compared with only 0.3% divergence within species (Fig. 1). At the nuclear *Tpi* locus, a 363 bp indel in intron 2 was found to differ between Highland Scotland and Southern English populations, with the inserted sequence present only in the Highlands. The two *Tpi* allelic types also differed at a number of fixed or nearly fixed sites, giving 1.8% divergence between northern and southern types, as compared with 0.5–0.6% divergence among alleles of the same type (Fig. 2). We scored populations for presence (long sequence) or absence (short) of the insertion at *Tpi*, as well as for the fixed sequence differences at cytB (Table 1).

The voltinism transition from two generations per year in the south to one per year in the north occurs to the south of the transition zone for mtDNA haplotypes (Fig. 3). Samples from many populations lying between the voltinism and mtDNA transition zones (and a few beyond) contained a mixture of southern and northern *Tpi* genotypes, or northern *Tpi* in specimens and populations containing southern mtDNA (Table 1, Fig. 3). In our samples, about half of the transitional populations in the North of England were fixed for *agestis* mtDNA haplotypes, the remaining being fixed for *artaxerxes* mtDNA; no mixed populations were found. The *agestis*-like mtDNA populations were also more likely to contain *agestis*-type *Tpi* alleles, although *artaxerxes Tpi* alleles were more common throughout the overlap zone. In North Wales, *agestis* mtDNA appeared to be fixed, but we found *artaxerxes Tpi* alleles in four populations, including both univoltine and bivoltine populations (Table 1).

Table 1. Collection sites and data summary for *Polyommatus* (*Aricia*) spp. in Britain. Values refer to numbers of individuals of each type, sampled in 1999 and 2000.

			mtDNA haplotype		*Tpi* intron 2 haplotype		
Site	Locality codes for Genbank	OS Grid Ref.	north	south	long (north)	short (south)	Gens. per year
Scotland (*A. artaxerxes*)							
Glen Fender	GFR	NN 8967	–	–	4	0	1
Creag Odhor	COR	NN 8863	–	–	2	0	1
Tomdhu	TOM	NH 9720	–	–	3	0	1
Shandwick	SHAN	NH 8573	–	–	1	0	1
Coupar Angus	LBC	NO 2734	(10)	(0)	2	0	1
Creag Dubh	CDH	NN 6795	–	–	2	0	1
Burn of Craig	BOC	NJ 4325	–	–	3	0	1
Spittal of Glenshee	SOG	NO 17	–	–	2	0	1
Pinbain Burn	PBB	NX 1391	–	–	2	0	1
William Hope	WHP	NT 4234	–	–	2	0	1
Northern England (overlap)							
Warton Crag (H)	WC	SD 4972	1	0	3	2	1
Gait Barrow	GBW	SD 4877	1	0	1	0	1
Whitbarrow	–	SD 48	(7)	(0)	–	–	1
Arnside Knott	–	SD 47	(10)	(0)	–	–	1
Smardale Gill	–	NY 70	(14)	(0)	–	–	1
Thrislington	TP	NZ 3132	4 (10)	0 (0)	10	0	1
Kettlewell	KET	SD 97	1	0	4	0	1
Cressbrook Dale (H)	CBD	SK 1773	0 (0)	3 (8)	4	2	1*
Coombs Dale (H)	CMD	SK 2174	(0)	2 (9)	4	1	1*
Lathkill Dale (H)	LKD	SK 1865	0	2	5	0	1*
Ellerburn Bank	ELB	SE 8584	0	5	0	2	1*
Ashberry (H)	ASH	SE 5685	0	3	5	0	1*
Cowlam	CWM	SE 96	0	6	0	3	1*
Fordon (H)	FDBC	TA 0475	(0)	7 (10)	1	3	1*
North Wales (overlap)							
Mariandyrys (H)	MD	SH 6081	–	–	1	3	2
Penmon Quarry	PQ	SH 6381	–	–	0	2	2
Great Orme	GOa	SH 7683	0	3	0	2	2
Marle Hall Woods	MHW	SH 8078	(0)	3 (9)	0	3	2
Llanwstenin	LN	SH 8279	–	–	0	2	2
Pennycordynn-bach	–	SH 9175	(0)	(10)	–	–	2
Bryn Meiriadog	BM	SJ 0172	–	–	2	0	2
Graig Tremeirchion	GT	SJ 0872	0	2	0	2	1
Gop Hill	GH	SJ 0980	–	–	0	1	1
Graig Fawr (H)	GF	SJ 0580	–	–	1	1	1
Lixwm (H)	LX	SJ 1771	0	1	1	1	1
Loggerheads	LOG	SJ 2063	–	–	0	1	1
Eryrys	EP	SJ 2057	–	–	0	2	1
Castle Woods	CW	SJ 1156	–	–	0	1	1
Southern & Eastern England (*A. agestis*)							
Kirby on Bain	KOB	TF 2361	–	–	0	4	2
Skippingdale	SKP	SE 8712	–	–	0	4	2
Whisby Nature Park	WNP	SK 9166	0	1	0	1	2
Santon Downham	SDM	TL 8288	–	–	0	4	2
Aldbury Nowers	AN	TL 3517	–	–	0	5	2
Holkham	HLK	TF 84	0	1	0	4	2
Therfield Heath	TH	TL 3440	0	3	0	3	2
Harmer Green	HARM	TL 2516	–	–	0	2	2
Daneway Banks	–	SO 90	(0)	(7)	–	–	2
Albury	ALB	SP 60	0	1	–	–	2
Dancers End	–	SP 90	(0)	(7)	–	–	2

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Table 1. *Continued.*

			mtDNA haplotype		*Tpi* intron 2 haplotype		
Site	Locality codes for Genbank	OS Grid Ref.	north	south	long (north)	short (south)	Gens. per year
Swyncombe Down	SWYN	SU 6791	0	1	0	2	2
Magdelen Hill	MAG	SU 5029	–	–	0	7	2
Beacon Hill	BCH	SU 6022	–	–	0	3	2
Detling	D	TQ 7959	–	–	0	2	2
Lydden Down	LD	TR 2745	–	–	0	2	2
Sandwich Dunes	SD	TR 3557	–	–	0	1	2
Braunton Burrows	BBW	SS 4635	–	–	0	3	2
Penhale Sands	PHS	SW 7756	–	–	0	3	2

(H) = hybrid populations having different geographic origins of mtDNA vs. *Tpi* haplotypes, or of mixed *Tpi* haplotype ancestry.
Numbers of mtDNA haplotypes in parentheses indicate additional records from Aagaard *et al*. (2002).
1* = Partial second generation, late in the year, reported in some years in the regions containing these sites (Asher *et al*., 2001).

Discussion

The results show a broad 150–200 km band of populations of *Aricia* in northern England and northeast Wales where a mixture of *Tpi* haplotypes are found (Fig. 3, Table 1). This occurs in precisely the area where intermediate colour patterns have been observed, i.e. in the northern part of the range traditionally considered part of *P. agestis*, where this normally bivoltine species is univoltine (Emmet & Heath, 1990; Dennis, 1992; Smyllie, 1996, 2010; Asher *et al*., 2001; Aagaard *et al*., 2002), or, as in N. Wales, where there are both univoltine and bivoltine populations (Wynne *et al*., 2003; Burke *et al*., 2005). In these populations, almost any combination of mitochondrial and nuclear haplotypes and voltinism may be present (Table 1). Most sites where mixed *Tpi* haplotypes are present have *P. agestis* mtDNA haplotypes, although some populations in NW England have *P. artaxerxes* mtDNA haplotypes (Warton Crag to Thrislington; Table 1).

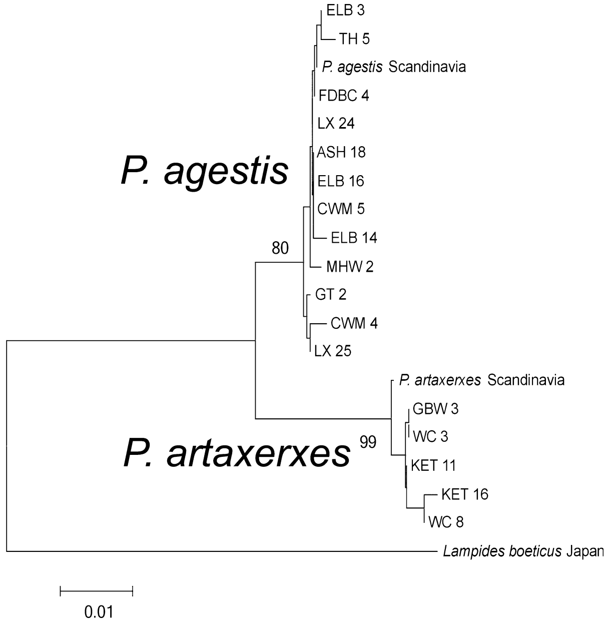

Fig. 1. Neighbour-joining tree of representative mitochondrial *cytB* sequences. The figure shows the strong differences among *artaxerxes*-type (lower branch on tree) and *agestis*-type (upper branch) haplotypes. The haplotypes labelled *agestis* and *artaxerxes* from Scandinavia are haplotypes 1 and 5 (respectively) from Aagaard *et al*. (2002). Letter codes for populations as in Table 1. The scale bar represents fractional divergence per base pair ('p-distance'), and figures alongside branches show bootstrap probabilities of those branches.

It seems most likely that hybridisation and mutual introgression between the two taxa explain these patterns. Allozyme analyses of *Tpi* had previously shown some evidence of nuclear/mitochondrial mismatch (Aagaard *et al*., 2002). However, in that study, the conclusion was only: 'whether or not the UK Peak District populations are of hybrid origin awaits a more detailed study.' The current data confirms the hypothesis, then considered unlikely, of introgression between the two species in Northern England, and extends the conclusion to North Wales where voltinism is variable (Wynne *et al*., 2003; Burke *et al*., 2005).

The timing of past introgression is unknown. Virtually all land currently occupied by 'pure' British *P. artaxerxes*, and much of that occupied by populations of hybrid origin lay under ice at the Last Glacial Maximum, around 20 000 years ago (Mangerud *et al*., 2004). Thus, the oldest likely date for the current position of the hybrid populations would be around 11 500 years ago, following rapid warming at the glacial-Holocene transition. The youngest possible date is limited by historical recording; the broad-scale distribution of *P. artaxerxes/agestis* populations in northern England and Wales has apparently been fairly stable for at least 100 years (Heath *et al*., 1984; Asher *et al*., 2001). These hybrid populations were geographically disjunct from 'pure' populations of both *P. artaxerxes* and *P. agestis* throughout the 20th Century (Heath *et al*., 1984; Asher *et al*., 2001), so little or no gene flow should have taken place during this period. We suspect that the populations of hybrid origin arose hundreds and quite likely thousands of years ago.

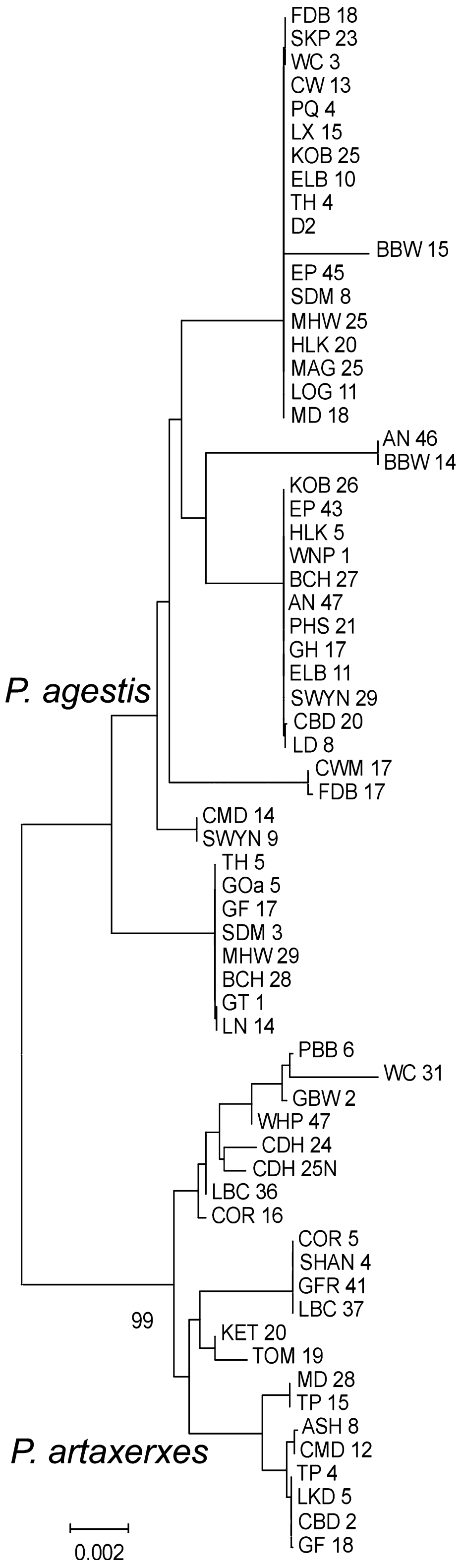

Fig. 2. Neighbour-joining tree of representative nuclear *Tpi* sequences. The figure shows diagnostic sequence differences within and among *artaxerxes* (long intron 2, lower branch on tree) and *agestis* (short intron 2, upper branch) sequences; the indel itself is excluded from tree reconstruction. Polymorphisms for northern and southern alleles are also shown within some populations (e.g. CMD, MD, WC). Letter codes for populations as in Table 1. The scale bar represents fractional divergence per base pair ('p-distance'), and the figure alongside the branch shows the bootstrap probability of that branch.

In view of our evidence for extensive past genetic interchange, with little sign of multiple locus bimodality in the transition zone, one could argue that the northern brown argus (*artaxerxes*) and brown argus (*agestis*) should again be considered conspecific under the traditional biological species concept: interbreeding between taxa has given rise to viable hybrid populations in Britain. A similar conclusion would result under a genotypic cluster delimitation because *artaxerxes* and *agestis* fail to show a bimodal distribution where they overlap in Britain (Jiggins & Mallet, 2000; Descimon & Mallet, 2009). On the other hand, *P. artaxerxes* and *P. agestis* populations are reported to remain distinct where they overlap in Jylland, Northern Denmark (Aagaard *et al*., 2002) and in Lithuania (Tolman & Lewington, 1997), suggesting that genotypic bimodality may occur in some contact zones. It is possible that the *P. artaxerxes* and *P. agestis* lineages vary in whether they meet biological species or genotypic cluster criteria for species in different geographic regions.

Those adopting a phylogenetic perspective on species (Cracraft, 1989) may in any case prefer to retain the two taxa as separate species, especially given the extensive differences between the pure forms in molecular haplotypes, *Tpi* alleles, voltinism, colour pattern, and host plant usage (e.g., Emmet & Heath, 1990; Dennis, 1992; Aagaard *et al*., 2002; Isaac *et al*., 2004). We make no firm recommendations for species delimitation here; instead, we argue that the focus of biodiversity conservation should be on endangered groups of organisms at any taxonomic level from populations to phyla, depending on biological distinctness. In the present case, it seems clear that *P. artaxerxes* in Britain is an endangered, morphologically, ecologically and genetically distinct taxon worthy of conservation interest, regardless of formal taxonomic status.

Taxonomically, and from the perspective of conservation designation, it is not possible to identify to which species each population belongs in the North Wales and Northern England zones of introgression because they contain a mixture of northern- and southern-origin genes. This appears inconvenient since the localised northern species, *P. artaxerxes*, is designated as a conservation priority (Biodiversity Action Plan species), whereas the more widespread and expanding southern species, *P. agestis*, is not. An alternative, which we favour, is to consider the introgressed populations a natural and probably ancient phenomenon. Thus the zone of introgression itself could be considered endangered (see below), and worthy of consideration for conservation. However, there may be some difficulty of achieving this under current legislation.

Bivoltine *P. agestis* in England have colonised northwards by approximately 200 km since around 1980, during recent regional warming (Asher *et al*., 2001; Thomas *et al*., 2001; Fox *et al*., 2006). The northernmost front of the expanding

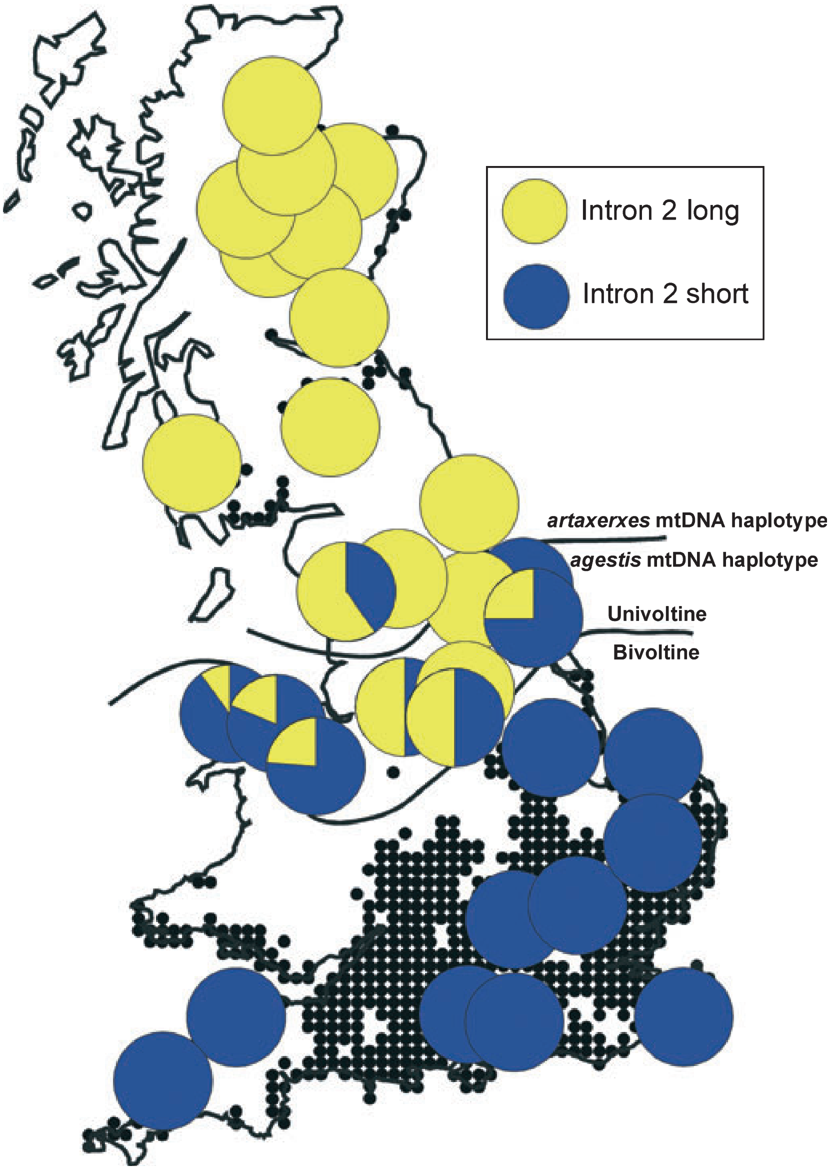

Fig. 3. Distribution of *Tpi* length variants for British populations of *Polyommatus* (*Aricia*). Pie charts show proportions of individuals from different populations with long (*artaxerxes*-type) and short (*agestis*-type) intron 2 lengths. The northern of the two lines shows the transition from *artaxerxes*-type mtDNA in the north to *agestis*-type mtDNA to the south. The southern line shows the transition in generation number; univoltine populations to the north of the line, and bivoltine to the south. Small black circles represent 10 km × 10 km grid Ordnance Survey grid squares with *Aricia* records.

bivoltine populations is now further north than the southernmost hybrid univoltine populations. Hybrid-origin and apparently pure *agestis* populations are now within a few tens of kilometres of one another. The older hybrid zone, which may date from relatively warm periods earlier within the Holocene, appears to be about to be re-activated. Southern bivoltine forms shifted northwards by 150 km in eastern England between 1970–1982 and 1995–1999 (Asher *et al.*, 2001), at a rate of 5–10 km per year. If this rate of spread continues, the whole area currently containing hybrid populations is likely to experience renewed introgression within another few decades, and there may be few if any 'pure' *artaxerxes* populations remaining in Britain by around 2100.

Considered from a global conservation perspective, the eventual loss of the northern species from Britain (perhaps with some remnant *artaxerxes* genes within predominantly *agestis*-like populations) is not necessarily a problem provided that *artaxerxes* is capable of surviving further north in Scandinavia or in high mountains elsewhere, where it already occurs. However, globally, there are many species restricted to localised areas (e.g., mountains) that could potentially suffer complete extinction in the face of increased introgression from warmer-adapted taxa. Were *P. artaxerxes* restricted to Britain (it is not), it might well be facing complete extinction through this process. In addition, the distinctive British form or subspecies, *P. artaxerxes artaxerxes*, characterised by a well-developed forewing medial white spot, will probably become extinct.

Recent conservation interest has placed considerable emphasis on attempting to increase rates of spread and assisted colonization to enable them to adjust their distributions to climate change (e.g., Hunter, 2007; Hoegh-Guldberg *et al.*, 2008), and this is potentially beneficial for habitat-restricted species whose expansions are thereby facilitated. However, the consequences for hybridisation are uncertain, and may include the passage of beneficial genes from one taxon to another, creation of a hybrid swarm, the formation of new hybrid species, or the elimination of one of the taxa. The last of these is a potential cause for concern. For species that experience increased introgression as a result of the expansion of related taxa, the consequences may be negative or even fatal. Helping *P. agestis* to spread northwards within Britain would likely speed the demise of *P. artaxerxes* within Britain. Thus, the development of biodiversity adaptation strategies in the face of climate change requires considerable care.

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Note

The molecular work described here was carried out by Ian Wynne. However, neither of the other two authors has been able to make contact with Wynne for several years, in spite of extensive efforts. Therefore, the data was analysed and written up by James Mallet and Chris Thomas on the basis of files left by Wynne on computer disks in Mallet's UCL laboratory. We publish this paper under Wynne's authorship because we believe that he would have wanted to see these novel findings made public in a forum such as this journal.

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