Interspecific and geographical variation in the sequence of rDNA expansion segment *D3* of *Ixodes* ticks (Acari: Ixodidae)

DENSON KELLY MCLAIN*, JING LI & JAMES H. OLIVER, JR

Biology Department, PO Box 8042, Georgia Southern University, Statesboro, Georgia 30460, U.S.A.

The base sequence of the rDNA *D3* expansion segment and flanking *H14* stem varies between six species of *Ixodes* ticks (Acari: Ixodidae) where only 33 invariant sites occur among sequences of 123–203 bases in length. Multiple copies of *D3* were sequenced from localities across the geographical ranges of four species to investigate deep population genetic structure. Two species, *I. pacificus*, from western North America, and *I. ricinus*, from Europe, have no sequence variation indicating a lack of deep genetic structure. One species, *I. scapularis*, from eastern North America has two forms of the *D3* sequence that are distributed differently among northern vs. southern populations, suggesting recent divergence and hybridization. *I. persulcatus*, from Eurasia, has sequence variation between localities of the order of that observed between other species, suggesting a long history of population isolation and deep genetic structure. With the exception of *I. scapularis*, sequence variation was not observed within localities. This indicates that cellular processes underpinning concerted evolution have homogenized populations and species for particular rDNA sequence variants.

Keywords: concerted evolution, *D3*, expansion segment, genetic structure, rDNA, tick.

Introduction

The population genetic structure of a species is the geographical distribution of its component demes and is inferred from both the spatial scale at which genotypes are observed to change and the evolutionary rates of the loci examined (Slatkin, 1987). Population genetic structure is deep if both slowly and rapidly evolving loci exhibit spatial variation and shallow if only rapidly evolving loci exhibit isolation by distance. Population genetic structure reflects historical events such as bottlenecks, colonizations, and range expansions and it reflects also the spatial scale at which the cohesive effect of gene flow is countered by microevolutionary forces promoting genetic divergence (Thompson, 1999). Gene flow rates are determined by habitat continuity, vagility of the species, and barriers to dispersal (Slatkin, 1987).

The population genetic structure of parasites may depend on host habitat preferences and host mobility (Mulvey *et al.*, 1991). Ticks (Acari) are ectoparasites of extremely limited intrinsic vagility (Adeyeye & Butler, 1989). Consequently, population genetic structure varies according to the host species utilized (Lampo *et al.*,

1998). In general, however, ticks exhibit shallow population genetic structure (Bull *et al.*, 1984).

In the present study, we examine population genetic structuring in four widespread species of hard ticks (Ixodidae): *Ixodes pacificus* (from western North America), *I. persulcatus* (from eastern Europe and Asia), *I. ricinus* (from Europe, western Asia, and northern Africa), and *I. scapularis* (from eastern North America) (Dennis *et al.*, 1998). As members of the *I. ricinus* complex, all are competent vectors of human diseases such as spirochaete-mediated Lyme disease (Lane *et al.*, 1991). By including several species in our analysis, we may be able to determine if there is a general pattern for tick genetic structure. A pattern would concomitantly suggest similar rates and mechanisms of gene flow and similar potential to vector disease agents across geographical ranges.

Prior studies employing rapidly evolving genetic markers have revealed shallow genetic structure in *Ixodes* ticks (Norris *et al.*, 1996; Kain *et al.*, 1999). We have chosen to use rDNA sequence data which could reveal deep genetic structure. Eukaryotic rDNA consists of arrays of a unit containing spacers and associated rRNA genes (Hillis & Dixon, 1991). The rate of sequence evolution varies across a repeating unit and even within a gene (Hillis & Dixon, 1991). This permits

*Correspondence. E-mail: dk_mclain@gsuvm2.cc.gasou.edu

the selection of particular sequences as markers for a desired level of phylogeographic resolution.

The eukaryote 28S rRNA gene is homologous to the prokaryote 23S rRNA gene except for the presence of about a dozen expansion segments ranging in length from 10 to several hundred base pairs (Hancock & Dover, 1988; Hancock *et al.*, 1988). Expansion segments evolve at up to 10 times the rate of the gene core (Kuzoff *et al.*, 1998).

Therefore, we chose to examine sequence variation in the approximately 150-base pair, AT-rich (usually) *D3* expansion segment which lies between highly conserved core sequences (Hancock & Dover, 1988). *D3* exhibits substantial intraspecific and interspecific sequence variation (Litvaitis *et al.*, 1994) which is phylogenetically informative and useful for the study of population structure (Kuzoff *et al.*, 1998). Between the conserved core sequences and each side of *D3* are 14 relatively nonconserved bases that together correspond to a stem, *H14*, in the folded transcript. These flanking sequences are also included in our analyses.

We also compare intraspecific sequence variation to interspecific variation among these and two other accessible species: *I. woodi*, which is widely distributed in North America west of the Mississippi River (Robbins & Keirans, 1992), and *I. affinis* (also a member of the *I. ricinus* complex) which occurs in Central America and south-eastern North America (Oliver *et al.*, 1987).

Materials and methods

Sources of ticks

Fertilized tick eggs, larvae, or adults were provided from field collections or laboratory stocks as indicated in Table 1. Live tissue samples were placed in isopropanol prior to shipping. Subsequently, samples were stored at -70°C until DNA was extracted. Species were represented by the following number of geographical samples: one for *Ixodes affinis*, three for *I. pacificus*, eight for *I. persulcatus*, six for *I. ricinus*, 10 for *I. scapularis*, and one for *I. woodi*. For each locality of each species, a single DNA extraction was performed on the entire (pooled) sample as listed in Table 1.

DNA extraction and PCR amplification

DNA was isolated using the procedure of Livak (1984) which entailed grinding tissues with plastic pestles in a lysis buffer of SDS that contained EDTA to chelate proteins. Enzymes in the slurry were heat denatured then precipitated on ice after addition of potassium acetate. Following centrifugation, the supernatant was

retained and ethanol precipitated to recover nucleic acids. One-2500th of each resulting 10 μg recovery of DNA was used to amplify rDNA *D3*. PCR amplification employed a Perkin Elmer DNA thermal cycler and Perkin Elmer GeneAmp reagents (Perkin Elmer Corporation, Norwalk, CT). DNA was amplified in a 100- μL volume for 37 cycles. The first cycle consisted of 1 min for denaturation at 97°C , 2 min for annealing at 37°C , and 2 min for extension at 72°C . The next 35 cycles were the same except that denaturation was at 94°C . The last cycle was the same as the middle 35 cycles except that extension was permitted for 10 min. Amplification primers were: (1) 5' GT GAATTC ACCCGTC-TTGAAACAC 3' with the 6-base *EcoRI* recognition sequence ending at base 8 and a conserved 28S sequence running from primer bases 9–25 (= bases 4045–4060 in Tautz *et al.* [1988]) and (2) 5' GT GGATCC TGAGG-GAAACTTCGG 3' with the 6-base *BamHI* recognition sequence ending at base 8 and a conserved 28S sequence running from primer bases 9–23 (= bases 4424–4410 in Tautz *et al.* [1988]). The 3' end of the primers represent conserved core sequences that flank the *D3* expansion segment in *Drosophila melanogaster* (Tautz *et al.*, 1988).

PCR products and the plasmid *pBluescript II* (Stratagene Cloning Systems, La Jolla, CA) were digested with both *EcoRI* and *BamHI*, ethanol-precipitated, resuspended in water, and ligated. *Escherichia coli* strain XL1-Blue (Stratagene) was transformed with the ligation products using the CaCl_2 procedure and ampicillin selection (Sambrook *et al.*, 1989).

DNA sequencing and analysis

Four subclones were sequenced from almost all sites from which a sample of a tick species was received. Two exceptions were *I. persulcatus* from Hokkaido Island (Japan) and Inner Mongolia (China) where only one subclone was sequenced from each site. Double-strand inserts in *pBluescript II* were sequenced by the dideoxy chain-termination method using Sequenase reagent kits (U.S. Biochemical Corporation, Cleveland, OH). Inserts were sequenced in both forward and reverse directions. When compressions were suspected, sequencing reactions were repeated using dITP. Sequencing reaction products were separated in a 6% acrylamide/urea gel for which the top and bottom buffers were 1 $\times$ TBE (Sambrook *et al.*, 1989). GenBank/EMBL accession numbers for sequences are provided in Table 1.

Sequence alignment was accomplished with GENETIC DATA ENVIRONMENT (Smith *et al.*, 1994). Phylogenetic analyses using the criteria of maximum likelihood and maximum parsimony were conducted with PAUP version 4.0b3 (Swofford, 2000). The neighbour-joining method (Saitou & Nei, 1987) was also used to assess

Table 1 Sources of ticks, nature of tissues used for PCR amplification, and GenBank/EMBL accession numbers for associated DNA sequences

Species and access no.	Locality	Tissue type	Collector
<i>Ixodes affinis</i> AF303991	South-eastern U.S.A. (Georgia)	3 adult males and 3 adult females	J. Hutcheson, Ft. Collins, CO
<i>Ixodes pacificus</i> AF303989 AF303989	South-western U.S.A. (Arizona) Western U.S.A. (2 sites: California)	3 adult males and 3 adult females Eggs of 6 females of a laboratory colony (established in 1992)	J. M. C. Ribeiro, Tucson, AZ J. H. Oliver, Jr., Statesboro, GA
<i>Ixodes persulcatus</i> AF303994 AF303992	Central Russia (Altai Mountains) Western Russia (near St. Petersburg)	Eggs of 5 field- collected females Eggs of 9 field- collected females	E. Korenberg, Moscow E. Korenberg, Moscow
AF303993	Western Russia (near Moscow)	3 adult males and 3 adult females	N. A. Filippova, St. Petersburg
AF303995	Eastern Russia (Sakhalin Island)	Eggs of 16 field- collected females	E. Korenberg, Moscow
AF303997	Dagestan	3 adult males and 3 adult females	N. A. Filippova, St. Petersburg
AF303999	Central China (Heilongjiang)	Larvae from eggs of field-collected females	Ai Cheng Xu, Beijing
AF303998	Northern China (Inner Mongolia)	Eggs of 3 field- collected females	Dou Gui-lan, Beijing
AF303996	Northern Japan (Hokkaido)	Eggs of a field- collected female	K. Miyamoto, Asahikawa
<i>Ixodes ricinus</i> AF303988	Czechoslovakia (Bratislava)	Eggs of 5 field- collected females	M. Labuda and F. Čiampor, Bratislava
AF303988	Ireland (County Wicklow)	Eggs of a field- collected female	J. Gray, Dublin
AF303988	Eastern Rumania (Moldova)	3 adult males and 3 adult females	E. Korenberg, Moscow
AF303988	Western Russia (Pskov region)	Eggs of 3 field- collected females	N. A. Filippova, St. Petersburg
AF303988	Switzerland (Neuchâtel)	Eggs of a field- collected female	L. Gern, Neuchâtel
AF303988	Thetford Forest (Norfolk, England)	3 adult males and 3 adult females	P. Nuttall, Oxford
<i>Ixodes scapularis</i> AF303986 AF303987 AF303987	Mid-eastern U.S.A. (2 sites: Maryland and North Carolina) Mid-western U.S.A. (2 sites: Minnesota and Illinois)	Nymphs of laboratory colonies (established in 1992) 3 adult males and 3 adult females from each	J. Piesman, Ft. Collins, CO J. Hutcheson, Ft. Collins, CO
AF303987	North-eastern U.S.A. (3 sites: Massachu- setts, New Jersey, and New York)	Nymphs of laboratory colonies (established in 1985, 1992, respectively)	J. H. Oliver, Jr., Statesboro, GA and J. Piesman, Ft. Collins, CO
AF303986 AF303987	South-eastern U.S.A. (3 sites: barrier islands off Georgia and Florida)	3 adult males and 3 adult females from each	J. Hutcheson, Ft. Collins, CO
<i>Ixodes woodi</i> AF303990	Central U.S.A. (Oklahoma)	3 adult males and 3 adult females	C. Hopla, Norman, OK

phylogenetic relationships. Genetic distances, d , were calculated using the method of Tamura (1992) which is less likely to underestimate d when d is small and when there are transition–transversion or base-composition biases.

To verify that *D3* and the supporting stem, *H14*, were subcloned, sequences flanking the putative expansion segment were compared to those of the *D3*-flanking, highly conserved core region of the rDNA 28S gene of *Drosophila melanogaster* (Tautz *et al.*, 1988). Flanking sequences of *Ixodes* species matched those of *Drosophila melanogaster* except for two base substitutions (5' ACCGTCTTGAAACACGGACCAAGGAG... CC-CGAAAGATA_TGGTGA_AACTATG_A 3', where subscripts indicate substitutions in *D. melanogaster* for the preceding base in ticks). The similarity confirmed the presence of *H14* and *D3* in *Ixodes* spp. subclones.

Results

Intraspecific variation: within populations

Sequence variation was not observed within localities of *I. pacificus* (three sites), *I. persulcatus* (eight sites), *I. ricinus* (six sites), *I. woodi* (one site), and *I. affinis* (one site). However, most *I. scapularis* sites from the south-eastern and mid-eastern U.S. had the same two sequence variants. To distinguish these, one is referred to as the scapularis form and the other as the dammini form (reflecting an *I. scapularis* clade formerly described as the separate species, *I. dammini* [see Rich *et al.*, 1995]). The scapularis form predominated in the south-eastern U.S. (2/4, 3/4 and 4/4 of sequences from three sites). In the mid-eastern U.S., the scapularis form was less common (1/2 and 2/4 of sequences from two sites).

Scapularis (190 bp) and dammini (188 bp) forms matched at 89% of their bases in the alignment that included *D3* and flanking *H14* sequences of all species. Transversions ($N=14$) distinguished the sequences more than did transitions ($N=5$) and insertions/deletions (= indels; $N=5$) (Table 2).

Intraspecific variation: between populations

No sequence variation was observed between localities for either *I. pacificus* (three sites, 12 sequences) or *I. ricinus* (six sites, 24 sequences). For *I. scapularis*, between-localities variation consisted only of differences in the frequencies of scapularis and dammini forms of the sequence (see above). The dammini form was present at all collection sites ($N=10$) except for one in the southeastern U.S. (Florida). In contrast, the scapularis form was not present in any of the northeastern or midwestern U.S. sites (five sites, 20 sequences). The

Table 2 Nature of sequence variation in *Ixodes* as a function of the level of comparison

	Within species		
	Within population*	Between populations†	Between species
No. transitions	4	37	39
No. transversions	12	54	34
No. indels of length			
1	3	17	27
2	0	10	13
3	0	2	5
4	0	1	4
5	0	2	2
6	0	0	2
7	0	0	1
>10	0	0	1

* *I. scapularis*: dammini vs. scapularis forms.

† *I. persulcatus* localities.

frequency of scapularis and dammini forms varied significantly among geographical regions (Kruskal–Wallis test, $\chi^2_3 = 8.46$, $P = 0.037$).

Extensive sequence variation occurred among eight *I. persulcatus* sites (26 subclones). The two sites in western Russia (Moscow, St. Petersburg) had identical sequences and varied from a south-central Russian site (Altai Mountains) by a single base. All other comparisons among sites were as different in base sequence as comparisons among different species (Fig. 1). Overall, there was a small but significant correlation between genetic and geographical distance (log-transformed) ($r = 0.40$, $F_{1,26} = 4.92$, $P = 0.036$; Table 3).

Alignment of sequences of all *I. persulcatus* populations (branch A of Fig. 1) resulted in an average level of sequence identity (base matching) of 81%. Sequence variation among *I. persulcatus* localities was almost equally due to base substitutions ($N=82$) and indels ($N=65$). The indels ranged from one to six bases in length and accounted for 96 base insertions or deletions (Table 2). Among base substitutions, transversions ($N=50$) were significantly more common than transitions ($N=32$; $\chi^2_1 = 3.95$, $P = 0.045$). Indels of only 1–2 bases were eight times as common as those of three or more bases (Table 2).

Extent of interspecific variation

The low degree of sequence variation observed within species (excepting *I. persulcatus*) contrasted with moderate to high levels of interspecific variation (Fig. 1). This variation yielded the same phylogenetic arrangement of *D3* sequences with both neighbour-joining

Fig. 1 Maximum parsimony phylogeny of *Ixodes* populations with percentage support for major features based on maximum likelihood (lower number) and maximum parsimony (upper number).

Table 3 Genetic and geographical distances separating *Ixodes persulcatus* populations

	Russia St. P.	Russia Moscow	Altai Mtns.	Sakhalin	Japan	Dagestan	Central China	Inner Mongolia
Russia St. P.	—	0	0.008	0.172	0.094	0.074	0.178	0.177
Russia Moscow	600	—	0.008	0.172	0.094	0.074	0.178	0.177
Altai Mtns.	3700	3500	—	0.172	0.084	0.074	0.178	0.177
Sakhalin	6200	6200	4400	—	0.088	0.173	0.235	0.262
Japan	7100	7100	4300	800	—	0.127	0.209	0.207
Dagestan	2100	1700	3500	7300	7700	—	0.150	0.140
Central China	6500	5800	2500	3300	3100	6300	—	0.024
Inner Mongolia	5600	5000	2300	2700	2900	5700	1000	—

Numbers above the diagonal are Tamura (1992) genetic distances. Numbers below the diagonal are geographical distances (km). Abbreviations: St. P., St. Petersburg.

and maximum parsimony analyses (Fig. 1). Further, maximum likelihood suggested the same relationships, except that the positions of *I. ricinus* and *I. pacificus*

were reversed. Thus, several major features of the phylogeny were well supported. First, all *I. persulcatus* populations clustered on the same major branch and in

isolation from other species. Second, the *I. affinis* sequence most resembled the inferred ancestral *I. ricinus* complex sequence. Third, both forms of the *I. scapularis* sequence shared more recent ancestry with each other than either did with any other sequence.

On average, sequences of species of branch B of Fig. 1 (*I. scapularis*, *I. pacificus*, *I. ricinus*, *I. woodi*) matched at 93% of their bases. The alignment of the *I. woodi* sequence (branch C of Fig. 1) with branch B species sequences yielded an average level of sequence identity of 83%. However, when *I. persulcatus* sequences were aligned with those of *I. woodi* or branch B *Ixodes* species, they matched at only 68% and 67% of their bases, respectively.

Nature of interspecific variation

Base substitutions ($N=112$) were more common than indels ($N=64$) among mutations accounting for interspecific sequence variation (Table 2). As with intraspecific variation in *I. persulcatus*, transitions ($N=50$) were less common than transversions ($N=62$) but here the difference was not significant ($\chi^2_1=1.29$, $P=0.173$). Indels of one to two bases were 2.2 times as common as those of three or more bases.

There was no difference in the relative numbers of indels and base substitutions depending on whether sequence variation was within or between species ($\chi^2_1=1.03$, $P=0.311$). Also, the number of transitions to transversions did not vary as a function of the source of sequence variation ($\chi^2_1=1.41$, $P=0.235$). The length of indels for within-species variation, which was dominated by *I. persulcatus*, was less than for between-species variation (Mann-Whitney $U=1700.50$, d.f. = 1, $P=0.011$; Table 2).

A number of indels characterized differences between *I. persulcatus* and all other *Ixodes* species. Sixteen gaps were shared among *I. persulcatus* sequences relative to their alignment with branch B *Ixodes* species (see Fig. 1). Gaps suggested deletions of 1–18 bp that encompassed a total of 56 bp. Relative to *I. persulcatus*, branch B species (*I. pacificus*, *I. ricinus*, *I. scapularis* and *I. woodi*) shared only three alignment gaps of 2–4 bases. These gaps encompassed a total of 10 bp. Thus, *I. persulcatus* sequences were on average 46 bp shorter than those of other *Ixodes* species.

Discussion

Population structure

D3 sequences did not vary geographically in *I. pacificus* or *I. ricinus*. These observations are likely to be a consequence of both (1) shallow (weak) population

structure and (2) the limited resolution of *D3* sequence variation in the assessment of intraspecific variation. For instance, mtDNA sequences revealed haplotype diversity within populations across the range of *I. pacificus* from British Columbia to the south-western U.S. (Kain *et al.*, 1999). However, the diversity exhibits no geographical pattern or structuring, except that samples of an isolated Utah population differed significantly from other localities (Kain *et al.*, 1999). Perhaps inclusion of additional sites for *I. pacificus*, such as from Utah, would have revealed some intraspecific variation in the *D3* sequence. Variation in rDNA *ITS 2* sequences also suggest only weak population structure for *I. pacificus* but, again, only a few sites were sampled (McLain *et al.*, 1995a). Weak geographical structuring (Kain *et al.*, 1999) is probably a reflection of host vagility. *I. pacificus* larvae, nymphs and adults parasitize over 80 species of reptiles, mammals and birds (Lane *et al.*, 1991).

The lack of deep structure in *I. ricinus* may also be explained by the vagility of its hosts. *I. ricinus* infests over 20 species of passerine birds, some with migratory routes that link southern and northern Europe (Mehl *et al.*, 1984). Some bird hosts show no geographical structuring of their breeding populations across northern and western Europe, consistent with long-range dispersal and geologically recent colonization (see Bensch & Hasselquist, 1999). Dispersal of *I. ricinus* ticks across Europe by birds could underpin the gene flow necessary to account for the observed absence of population structure. Also, much of the current range of *I. ricinus* was glaciated during the most recent Pleistocene ice ages (see Jaarola *et al.*, 1999). Recent range expansion of *I. ricinus* northward from southern peninsular refugia appears, as is the case with much of the European biota, to be associated with a low degree of genetic diversity and concomitant absence of deep genetic structuring (Hewitt, 1999).

Geographical structuring was indicated for *I. scapularis*. The range includes much of North America east of the Mississippi River. Here, a southeastern population is indicated by the presence of a *D3* sequence variant not observed in the northeastern or midwestern parts of the range. This observation is consistent with the presence of two major clades that have been posited on the basis of variation in mitochondrial 12S and 16S rDNA sequences (Rich *et al.*, 1995; Norris *et al.*, 1996). Analysis of rDNA *ITS 1* sequences (McLain *et al.*, 1995b) and mtDNA *cytochrome b* SSCPs (Mixon, 1999) also suggests similar genetic differentiation in *I. scapularis*.

Unfortunately, the present analysis does not identify the nature of sequence variation in *I. scapularis*. The different *D3* sequences may co-occur in tandem arrays of rDNA repeating units. Or haploid genomes may be

homogeneous for either variant. In this case, some populations contain an admixture of such genomes but in different proportions. The latter alternative is consistent with hybridization between members of northern and southern clades (see Hutcheson *et al.*, 1995). Hybridization could be a consequence of range expansions over the last 50 years (Wilson, 1996). Uniformity of haploid genomes is also consistent with high rates of homogenization within and between rDNA arrays (Schloetterer & Tautz, 1994; see below). For instance, the absence of within-population variation in *I. persulcatus*, in spite of substantial between-population variation, suggests that repeats within an array share common ancestry more recently than do ticks from different populations.

Monophyly of *I. persulcatus* populations is strongly supported (Fig. 1) and revealed in shared deletions of approximately 60 bases. Yet, *I. persulcatus* from different localities exhibit as much sequence variation in the *D3* as is observed between all other *Ixodes* species. Thus, population structure is deep.

Geographical proximity explains some of the genetic structure observed in *I. persulcatus*. For instance, sequences from nearby localities in western Russia were identical. Relative geographical proximity is also associated with sequence similarity between China and Inner Mongolia and between Sakhalin and Japan. These associations suggest genetic cohesion by way of host-mediated gene flow. Yet, sequences from the Altai Mountains were almost identical to those from western Russia (≥ 3500 km apart). This probably reflects the dispersal and population structure of hosts. For instance, lemmings show no population structure throughout their postglacial range that encompasses arctic Eurasia (Federov *et al.*, 1999).

Deep structure in *I. persulcatus* may reflect the past presence of multiple refugia from glaciers (including central China as a refuge). Independent evolution of sequences may have occurred within individual refugia before recolonization of the postglacial range (see Hewitt, 1999).

Hosts and dispersal

Host specificity varies among species of ticks (Hoogstraal & Aeschlimann, 1982). However, all four of the *I. ricinus* complex species parasitize various species of reptiles, mammals and birds (James & Oliver, 1990; Lane *et al.*, 1991). Reptiles generally exhibit moderate to deep genetic structure across small spatial scales due to limited dispersal and habitat discontinuity (e.g. Prosser *et al.*, 1999). Among mammals, bats and rodents are common hosts of some ticks (Hoogstraal & Aeschlimann, 1982). Bats have deeply structured populations

(e.g. Wilmer *et al.*, 1999) whereas rodents do not (e.g. Federov *et al.*, 1999). The genetic structure of birds varies among species (Haig *et al.*, 1997) but frequently reveals high rates of gene flow and long-range dispersal (e.g. Da Silva & Granadeiro, 1999). Thus, birds appear to offer the greatest potential for the long-range dispersal of ticks that would reduce genetic structuring across the range. Birds are frequent hosts of *I. pacificus* (Lane *et al.*, 1991), *I. persulcatus* (Anastos, 1957), *I. ricinus* (Mehl *et al.*, 1984), and *I. scapularis* (Klich *et al.*, 1996).

Concerted evolution

A general feature of rDNA sequences is that variation within species is reduced or absent even when interspecific variation is abundant (Hillis & Dixon, 1991). This represents the homogenization of the multigene family for a particular sequence variant (Dover, 1982). Homogenization is generally believed to result from unequal exchange (Schloetterer & Tautz, 1994) and biased gene conversion (Hillis *et al.*, 1991) and may occur at different rates for different regions of the rDNA repeating unit (Linares *et al.*, 1994; Polanco *et al.*, 1998).

Concerted evolution is the non-independent evolution of copies of a repeated sequence that results from homogenization (Arnheim, 1983). Concerted evolution of rDNA is rapid (Polanco *et al.*, 1998) which reduces allelic variation within demes and among localities united by gene flow (see Schloetterer & Tautz, 1994).

Our data suggest that *D3* sequences are rapidly homogenized, which would leave little evidence of within-population variation. Our results also suggest that *D3* sequences have been homogenized repeatedly. This would lead to the accumulation of interspecific variation with the same kinds of mutations that characterize within-species variation. Sequence identity among the observed *I. ricinus* complex species is 60% when *I. persulcatus* is excluded and only 12% when *I. persulcatus* is included. Thus, high rates of mutation and recurring episodes of homogenization have captured both the evolutionary independence of these species in highly characteristic sequences and the general evolutionary non-independence of populations composing each species.

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