

SHORT NOTE

Lack of nucleotide variability in a beetle pest with extreme inbreeding

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Abstract

The coffee berry borer beetle *Hypothenemus hampei* (Ferrari) (Curculionidae: Scolytinae) is the major insect pest of coffee and has spread to most of the coffee-growing countries of the world. This beetle also displays an unusual life cycle, with regular sibling mating. This regular inbreeding and the population bottlenecks occurring on colonization of new regions should lead to low levels of genetic diversity. We were therefore interested in determining the level of nucleotide variation in nuclear and mitochondrial genomes of this beetle worldwide. Here we show that two nuclear loci (*Resistance to dieldrin* and ITS2) are completely invariant, whereas some variability is maintained at a mitochondrial locus (COI), probably corresponding to a higher mutation rate in the mitochondrial genome. Phylogenetic analysis of the mitochondrial data shows only two clades of beetle haplotypes outside of Kenya, the proposed origin of the species. These data confirm that inbreeding greatly reduces nucleotide variation and suggest the recent global spread of only two inbreeding lines of this bark beetle.

Keywords: inbreeding, nucleotide variability, *Resistance to dieldrin*, mitochondrial DNA, coffee berry borer, *Hypothenemus hampei*.

Introduction

The coffee berry borer, *Hypothenemus hampei*, is the major insect pest of coffee and has spread to most of

the coffee-growing countries of the world (Le Pelley, 1968). This insect has also recently evolved resistance to the insecticide endosulfan (a cyclodiene type compound) (Brun *et al.*, 1989, 1990), which is one of the main chemicals used in control of the borer. As well as being a remarkably successful insect pest, *H. hampei* also displays an unusual life cycle with regular inbreeding. Simple population genetic theory suggests that this regular inbreeding and the founder effects occurring on the colonization of new regions should lead to low levels of genetic diversity. We were therefore interested in documenting the levels of nucleotide variability in this inbreeder and in trying to reconcile the expected reduction in variability with the ecological success of the insect and its ability to evolve insecticide resistance.

The coffee berry borer shows regular inbreeding. Single mated female *H. hampei* burrow into coffee berries and produce large single families with highly distorted sex ratios (ten females to one male). The dwarfed males are flightless and mate with their sisters in the natal berry. The beetle is also 'functionally haplodiploid', as paternal genes in males are neither expressed nor transmitted (Brun *et al.*, 1995a, b). Thus, both the nuclear and mitochondrial genome are strictly maternally inherited and should share an identical genealogy. Resistance to the cyclodiene type insecticide endosulfan has also recently been documented in *H. hampei* in the South Pacific island of New Caledonia (Brun *et al.*, 1989, 1990). Resistance is associated with replacement of a single amino acid, alanine302 > serine, in the γ -aminobutyric acid (GABA) receptor subunit coded for by the *Resistance to dieldrin* gene (ffrench-Constant *et al.*, 1994).

Here we report that both the cyclodiene resistance gene *Rdl* itself and a different nuclear locus, the Intergenic Spacer region (ITS2) of the 5.8S and 28S ribosomal RNA, show a complete absence of nucleotide variability across the globe (with the exception of the resistance associated point mutation in *Rdl*). Although a mitochondrial locus, cytochrome oxidase I (COI) retains some nucleotide variation. Phylogenetic

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analysis of this mitochondrial DNA variation suggests that only a few inbreeding strains of the coffee berry borer have colonized the world. The factors likely to reduce nucleotide variation in this inbreeder and the contrast with the remarkable ecological success of this beetle are discussed.

Results and Discussion

Lack of nuclear nucleotide variation

To test whether or not the level of nucleotide variation in *H. hampei* is reduced by inbreeding we examined two nuclear loci (*Rdl* and ITS2) and one mitochondrial locus (COI) via PCR amplification and direct nucleotide sequencing of the PCR products. We sequenced all three loci in seventeen strains collected world-wide. Sequencing of 800 bp from the insecticide resistance associated locus *Resistance to dieldrin* (ffrench-Constant *et al.*, 1994) (Fig. 1a) revealed no nucleotide variation, except for the presence or absence of the insecticide resistance associated nucleotide substitution. To guard against the possibility that this locus was under abnormally strong selection (although resistant strains have only been collected from the South Pacific island of New Caledonia) we also examined ITS2, a non-coding region of a second nuclear locus highly variable in other insects (Fig. 1b). Sequencing of 747 bp from ITS2 of all strains also revealed no variation.

Amplification and sequencing of both *Rdl* and ITS2 from the related inbreeding beetle *H. obscurus*, showing 1.9% and 4.0% divergence respectively from *H. hampei*, was carried out to guard against unintended repeated PCR amplification of exactly the

same product. We also sequenced 1203 bp from the *H. hampei* mitochondrial locus COI (Fig. 1c) and found twenty-one variable positions (1.8% variation) within populations. This higher level of variation in the mitochondrial genome can be explained by an apparent rapid rate of evolution (higher mutation rate) in *H. hampei* mitochondrial DNA. Thus, the open reading frame of the mitochondrial COI locus of *H. hampei* and *H. obscurus* shows a very high level of divergence (Ks, 0.84), whereas intron sequence from the nuclear locus *Rdl* diverges by only 2%.

Factors contributing to reduction in nucleotide variation

Several factors associated with different insect life cycles might be expected to reduce genetic polymorphism. In aphids, for example, these include parthenogenicity (anholocycly), annual population bottlenecks and founder effects (Loxdale & Brookes, 1989). Thus in aphids average biochemical polymorphism (P) is reduced, as is average heterozygosity (H) with values of approximately 4.5% against an average of 7.3% in other insects (Loxdale *et al.*, 1985). However, two factors relating to these studies are relevant to the current discussion. Firstly, biochemical polymorphism is maintained in aphids even in the presence of anholocycly. Secondly, many studies of variability in insects have been restricted to allozyme analysis and the level of underlying nucleotide variability has not been assessed.

The aim of this study was therefore to quantitate the reduction in nucleotide variation associated with the unusual life cycle of the coffee berry borer. Several factors associated with the life cycle of *H. hampei* would be expected to reduce nucleotide variation. (1) Only single families are usually found in each coffee berry. Full-sib mating would therefore be expected to reduce heterozygosity at a rapid rate. Calculations show a rapid initial decline in heterozygosity over initial generations (values reducing as follows for each generation; 1.0, 1.0, 0.75, 0.62, 0.50, 0.40, 0.32, 0.14, 0.04, 0.01 and 0.002) followed by an average of a 19% loss at each subsequent generation (Wright, 1921). Such a decline would dramatically reduce heterozygosity and full-sib mating may therefore be the dominant factor in reducing nucleotide variation in *H. hampei*. (2) Bottlenecks occurring upon colonization of new regions. As the spread of *H. hampei* to many countries has been relatively recent, little divergence within these founder populations would be expected. This factor would be expected to affect both mitochondrial and nuclear genomes. Further, as a result of both the level of inbreeding and population bottlenecks, as a particular mutation fixes in a population, variants present in the genome in which it arose will also be fixed, as there is no outcrossing. (3) Finally, the appearance of deleterious mutations will also elimi-

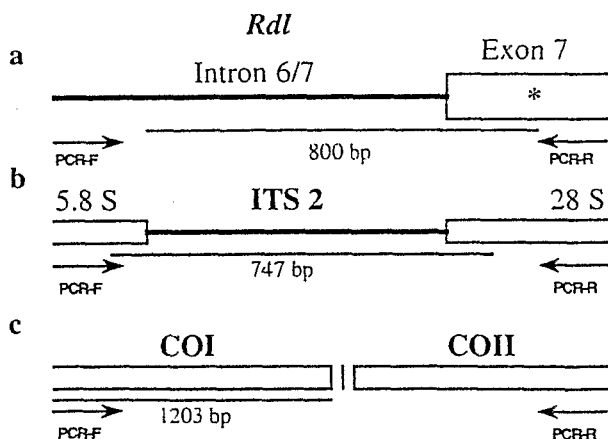


Figure 1. Diagram showing the relative location and lengths of the regions sequenced in the two nuclear loci (a) *Resistance to dieldrin* (*Rdl*, DDBL/EMBL/GenBank accession number AF037324) and (b) the second Intergenic Spacer (ITS2) of the two ribosomal RNA genes (accession number AF037326), and (c) the single mitochondrial locus cytochrome oxidase I (COI, accession number AF037325). A star indicates the location of the resistance associated mutation in *Rdl*.

nate the genomes in which they are found via natural selection, also reducing diversity. Our results on *H. hampei* thus confirm the predicted reduction in the nucleotide variability of this beetle, data also supported by the apparent lack of variation in a global survey of sixteen allozymes (Breilid & Kirkendall, unpublished).

Interestingly, despite the apparent absence of nucleotide variation in the two nuclear loci sequenced, the resistance associated point mutation in *Rdl* was present and has been selected for at least in the South Pacific island of New Caledonia (French-Constant *et al.*, 1994). If resistance is absent from the rest of the world (a systematic survey has not been completed), this may represent an independent origin of resistance in a single inbreeding line. However, regardless of the uniqueness of resistance in New Caledonia, the level of inbreeding in *H. hampei* will constrain independent

Global colonization by a few inbreeding lines

Examination of the most parsimonious tree of the mitochondrial data (Fig. 2) suggests that outside of Kenya, the putative ancestral origin of the species, the world has been colonized by only two inbreeding lines of *H. hampei*, one encompassing central and southern America and the other all strains from south east Asia, the south Pacific, Jamaica and the Ivory Coast. Two further factors are of interest in relation to the mitochondrial variability. Firstly, on a geographical level, the particular strain sequenced from Kenya does not correspond to either of the two colonizing clades. This may reflect a greater mitochondrial haplotype diversity in the source country. However, this assumption would have to be supported by analysis of a larger number of strains from Kenya. Secondly, given the unusual chromosome/life cycle of *H. hampei*, in which both the

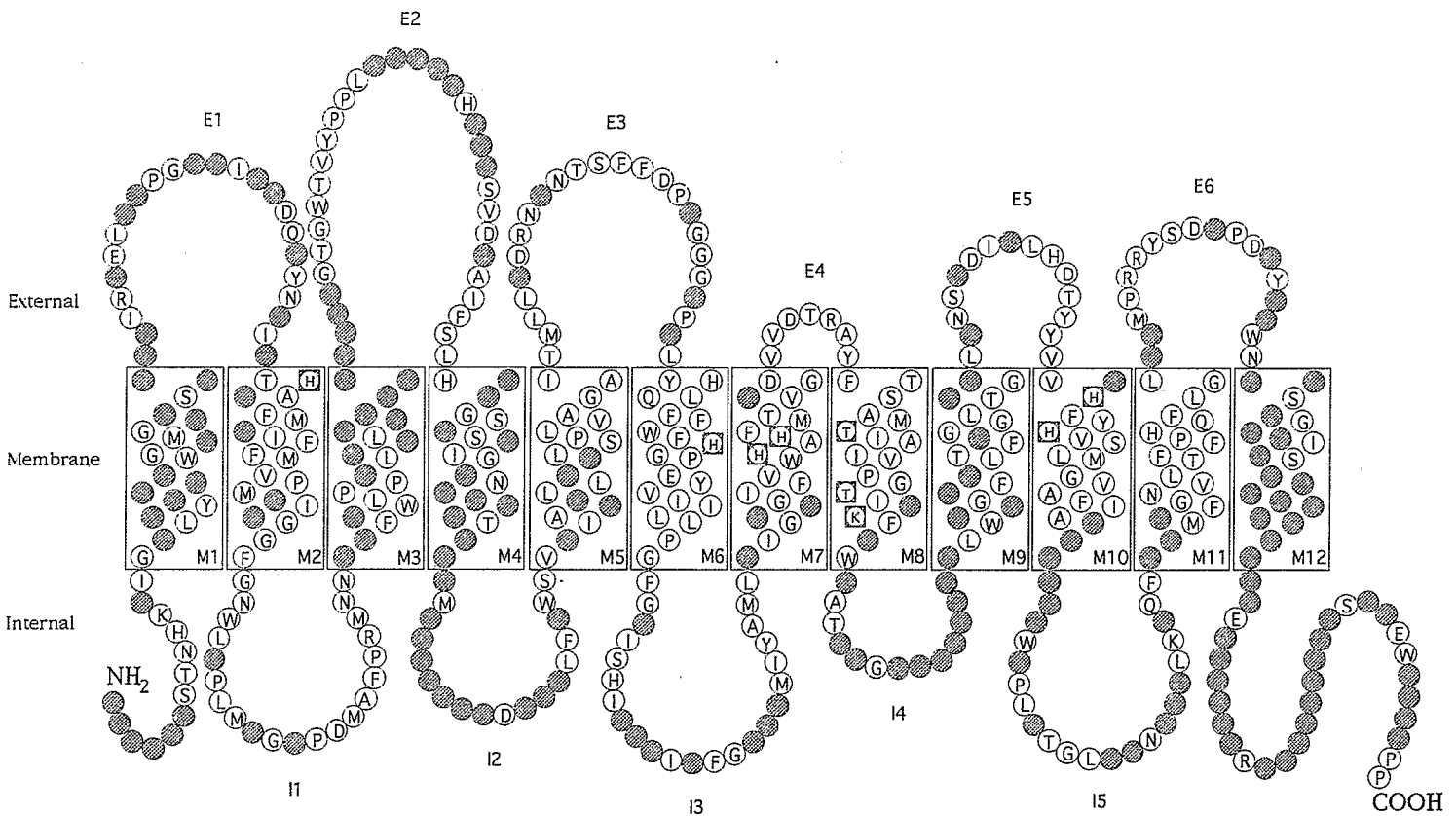
tained by independent selection of the two different mitochondrial haplotypes. The possibility of the mitochondria being heteroplasmic (more than one haplotype per individual) can be excluded as no heterozygosity was apparent in the sequencing ladders

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