Chapter 8

Patterns of hemoglobin polymorphism [α-globin (HBA) and β-globin (HBB)] across the contact zone of two distinct phylogeographic lineages of the European rabbit (*Oryctolagus cuniculus*)

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Abstract
Two loci, HBA and HBB, were studied for protein polymorphism across the contact zone of the European rabbit (*Oryctolagus cuniculus*). Six alleles were identified in HBA and two in HBB. Three alleles at the HBA locus were found to be restricted to some populations, while the other three revealed more broad geographic structure. An apparent substitution of the three major alleles in HBA along an Iberian southwest-northeast axis is proposed to be related to the two formerly described population units, including a hybrid allele within their contact zone. The two alleles of HBB are present in almost all populations at similar frequencies, obscuring the relationship between the two evolutionary units. The starkly contrasting pattern of allelic distribution among populations at these two loci – within a well-established bi-lineage phylogeographic framework – strongly suggests that non-neutral evolutionary processes are involved at a large scale.

Keywords: hemoglobin, hybrid allele, cytonuclear disequilibrium, contact zone, Iberia

Introduction

The European rabbit, *Oryctolagus cuniculus*, originated in southern Iberia but is currently distributed throughout the world, primarily due to human mediated introductions (Flux 1994; Callou 2003). Its well described history and phylogeographic structure offer an excellent framework within which one can pursue more specific population genetic questions directed toward the further understanding of the evolutionary dynamics of the species.

Within the Iberian Peninsula, two distinct groups of populations were identified using protein (Ferrand 1995; Ferrand & Branco, this volume) and immunoglobulin (van der Loo *et al*. 1999) polymorphisms, and these groups correspond to the designation of two subspecies, *O.c. algirus* (southwest

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Iberia) and *O.c. cuniculus* (northeast Iberia). Allele frequency variation of protein markers supported an estimate of 250 000-500 000 years divergence between the two population groups and further showed a large contact zone bisecting the Peninsula along a southwest-northeast axis (Branco 2000; Ferrand & Branco, this volume). An RFLP analysis of the whole rabbit mitochondrial DNA unveiled the existence of two highly divergent lineages, referred to as A and B. Lineage A was observed in southern Spain and lineage B in northern Spain, two French localities (Camargue and Versailles) and in domestic breeds (Biju-Duval *et al*. 1991). The same work provided a 2 myr divergence estimate between the two maternal lineages, a much greater estimate than that based on nuclear genes. More recently, RFLP data of the mtDNA cytochrome *b* gene gave a detailed view of the geographic distribution of these mitochondrial lineages (A and B) showing a narrower region of contact than that seen with protein markers (Branco *et al*. 2000). A strong correlation between both sets of genetic markers and the geographic distribution of the two subspecies was shown (Branco 2000), as well as cytonuclear disequilibrium (van der Loo *et al*. 1999). Such genetic structure reflects a long period of isolation in two refugial areas throughout the Pleistocene epoch. During the most recent interglacial, rabbits are thought to have expanded from these two refugia and subsequently formed the secondary contact zone in central Iberia (Branco *et al*. 2002). This scenario is concordant with similar patterns for other organisms, which demonstrate that the Iberian Peninsula was one of the major refuge areas for many southern European species (Webb & Bartlein 1992; Hewitt 1996; Myers *et al*. 2000; see also Gomez & Lunt, this volume).

The domestication of the European rabbit is recent, and may have resulted from a single event. Data from immunoglobulins (van der Loo *et al*. 1999), proteins (Ferrand 1995), mtDNA and microsatellites (Queney *et al*. 2002) indicate that all domestic breeds originate from the genetic pool available in France and only two variants of the B haplogroup were observed. Though a number of studies focused on allozyme variation in wild rabbit populations (e.g. Ferrand *et al*. 1988; Vieira & Ferrand 1995; Branco *et al*. 1998; Branco & Ferrand 2002), only more recent studies have been carried out in the context of the rabbit's evolutionary history (Branco *et al*. 1999; Branco & Ferrand 2003; Ferrand & Branco, this volume). We chose to study rabbit hemoglobin genes (HB), α-globin (HBA) and β-globin (HBB), based on the availability of samples (hemoglobin is the most abundant protein in blood), the extensive knowledge of its evolution and regulation (Hardison 1991, 2001) and the description of polymorphism in domestic rabbit (Ferrand 1989, 1990). The relationship of these two genes is additionally interesting as each contributes two polypeptide chains to the hemoglobin molecule but are located on different chromosomes and in a radically different genomic context: HBA has been assigned to chromosome 6 (Xu & Hardison 1991) and HBB to chromosome 1 (Xu & Hardison 1989).

A considerable amount of research has been carried out on rabbit HB, but almost exclusively focused on domestic animals (see review in Hardison 1991).