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Evolutionary genetics of early colonisation in Caribbean Anolis

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Evolutionary genetics of early colonisation in Caribbean *Anolis*

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by

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ABSTRACT

Despite the importance of the colonisation phase for invasion success, few early-stage introductions have been characterised and studied from the earliest stages following their initial importation. This thesis attempts to redress this information gap, using molecular techniques and common garden experiments to reconstruct colonisation history, reveal genetic diversity and demonstrate evolutionary potential in two recent introductions of Caribbean *Anolis*. Both of these colonisations provide useful models because they are examples of recent anthropogenic introductions on isolated land masses which contain congeneric native competitors. The colonisations studied in this thesis are the introduction of the Puerto Rican *Anolis cristatellus* to Dominica around 1998-2000, and of the Cuban native *A. sagrei* in St. Vincent around 2003.

Bayesian phylogenetic analysis of mtDNA sequence data was used to approximate the geographical origin of the introduced populations (*A. cristatellus* from north central Puerto Rico, and *A. sagrei* from Florida). Multiple mitochondrial haplotypes were present in Dominica, suggesting that at least seven reproductive females were present in the founding propagule, whilst in St. Vincent only a single haplotype was present. These approximate estimates of propagule size were used to evaluate the potential impact of demographic bottlenecks on genetic diversity and establishment success within introduced populations. Microsatellite analyses (using *F*-statistics and Bayesian methods) of the genetic diversity within and between native, source and introduced populations of *A. cristatellus* provided limited evidence for a genetic founder effect in Dominica. A replicated common garden experiment indicated that the variation in phenotypic characters amongst *A. cristatellus* populations at different altitudes on Dominica was predominantly genetically based, supporting the hypothesis that the introduced population is rapidly responding to novel environmental selection pressures. The findings of the study are discussed in relation to the differing establishment success of the two colonisations, and are related to the wider context of colonisation theory and management practice.

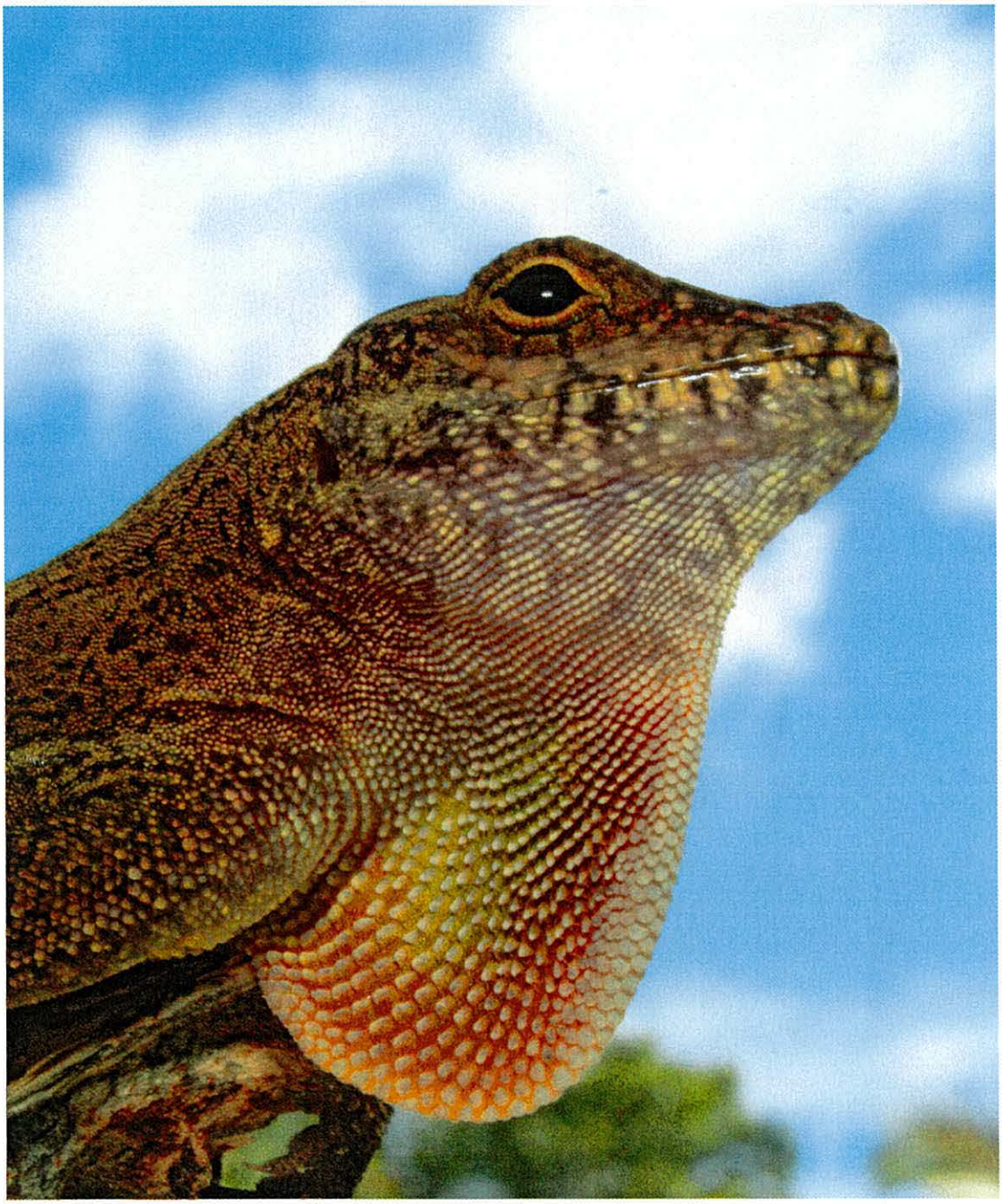

Plate I.

Anolis cristatellus from Botanical Gardens, Dominica, with extended dewlap.

Photograph by R.S. Thorpe

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PREFACE

Chapter 1 provides a thorough background to the island system and genus of study. A review of the theory and techniques is presented, annotated with empirical examples. The three experimental chapters are presented in the form of published papers. Chapter 3 has been published and is presented in Appendix V. Chapters 2 and 4 have been submitted for publication and are pending reviewer and editorial decisions. Chapter 5 contains a general discussion of the findings from the experimental chapters, relating the findings to genetic and evolutionary theory. The utility of the molecular methods used in this thesis are explored for the field of invasion biology. The implications of the findings in the wider context of species introductions is discussed.

Chapter 2 Eales J, Malhotra A and Thorpe RS (2008) Rapid adaptation of an invasive population: *Anolis cristatellus* in Dominica *(Submitted to Evolution)*

Chapter 3 Eales J, Thorpe RS, Malhotra A. (2008) Weak founder effect signal in a recent introduction of Caribbean *Anolis*. *Molecular Ecology* **17**, 1416–1426

Chapter 4 Eales J & Thorpe RS (2008) Significance of geographic origin, genetic diversity and stochasticity on the establishment potential of recently introduced *Anolis* in St Vincent, West Indies *(Submitted to Biological Invasions)*

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CHAPTER 1

General Introduction

1.1 Species introductions

1.1.1 Species introductions: definition and significance

Movements of biota outside their native ranges are a natural colonisation process that have contributed to faunal accumulations throughout the Earth's history. Contemporary examples of such naturally-mediated introductions are rare, although one case is the isolated sighting of the red-necked parrot, *Amazona arausiaca* (endemic to Dominica) in Guadeloupe (Evans & James 1997) mediated by Hurricane David in 1979. Green iguanas (*Iguana iguana*) were washed ashore on Caribbean beaches after 'rafting' on debris resulting from Hurricane Louis in 1995. This over-water dispersal was reported to be responsible for the colonisation of Anguilla by this species (Censky *et al.* 1998). Species introductions describe situations in which imported propagules of non-natives are able to establish self-sustaining populations outside the habitats where they evolved or spread naturally. The past century has seen a rapid increase in commercial anthropogenic movements on local, national and global scales, which has led to an associated dramatic rise in the incidence and geographical range of imported non-natives (reviewed in Lodge 1993). Species introductions are termed invasive if the non-natives adversely affect the habitats they invade economically, environmentally or ecologically (Colautti *et al.* 2004). Biological invasions are widely recognised as a significant component of global change (Vitousek *et al.* 1996), and a number of dramatically successful invasions are the direct result of deliberately transported non-natives (for example, mongoose *Herpestes spp.* in Pacific islands Case & Bolger 1991, Watari *et al.* 2008; *Rhododendron ponticum* in the British Isles, Turner & Watt 1939, Cross 1973, Milne & Abbott 2000; Cane toad *Bufo marinus* in Australia, Burnett 1997, Leblois *et al.* 2000, Lever 2001). Invasive species are estimated to cost 138 billion dollars annually in the United States of America alone (Pimentel *et al.* 2000), and between 50 and 80% of endangered or extinct species are estimated to be the direct or indirect consequence of introduced non-natives (Sakai *et al.* 2001).

Species introductions provide an excellent opportunity to investigate the drivers of ecological and evolutionary processes, thus are important for understanding the consequences associated with recent anthropogenically-induced environmental change. Studies of non-native species provide insights into the relative importance of competition, predation, and community complexity and stability for the dynamics of community assembly (e.g. Moulton & Pimm 1983, 1986). Introductions also provide a useful model for understanding the evolutionary consequences of demographic bottlenecks faced by many endangered species and fragmented populations (Frankham *et al.* 2002), and can contribute to the understanding of natural colonisation and radiation processes. The knowledge acquired through investigations of these ecological and environmental processes can be fed back into strategies to manage the impact of introduced and invasive species. The recent range expansion of non-natives and their ability to infiltrate previously uninhabitable areas are attributed not only to increased human-mediated transportation, but to the current global issues of climate change, habitat fragmentation and associated declining biodiversity. The social, economic and political concerns surrounding these environmental pressures highlights the importance of introduction studies in understanding of a variety of anthropogenic impacts on the Earth's biodiversity and ecosystem functioning.

Although the transportation of global commodities continuously exports floral and faunal propagules outside their natural ranges, a relatively small proportion of imported propagules successfully establish in novel environments (Shigesada & Kawasaki 1997). The "tens rule" (Williamson 1996), states that approximately 10% of propagules imported are seen in the wild, 10% of these become established, of which a further 10% become invasive due to their negative biological and ecological effects. The rule was originally described for British animals and plants, but is also applicable to many other introduction and invasion situations (e.g. pasture plants in the Northern Territory, Australia, Lonsdale 1994; non-indigenous molluscs in the United States of America, OTA 1993), with some notable exceptions (predominantly deliberate introductions, reviewed in Williamson & Fitter 1996). Thus, the fate of species introductions varies greatly and the factors which determine the likelihood of species establishment and invasiveness are complex and highly interrelated. Furthermore, invasive species with well-documented invasion histories are scarce (Suarez *et al.* 2001). For the vast majority of natural cases, it is difficult to identify source populations, colonisation

history and invasion routes. Consequently, elucidating mechanisms behind invasion patterns and the consequences of introduced species have been a recurrent challenge for investigations into invasion biology.

1.1.2 What factors facilitate introductions?

The likelihood of establishment of a non-native is determined by three main elements; the size of the founding population, the ability of the species to establish, and the susceptibility of the environment to introductions (Crawley 1986). These elements and the factors that contribute to them are represented in the diagram shown in Fig. 1.1. The interaction and relative importance of these influences culminates in the variety of ecological and evolutionary impacts that introduced species have on the communities and ecosystems they permeate. The identification of potentially invasive species, and of the environments susceptible to introduction are therefore of significant practical interest for preventing invasions and minimising their detrimental effects.

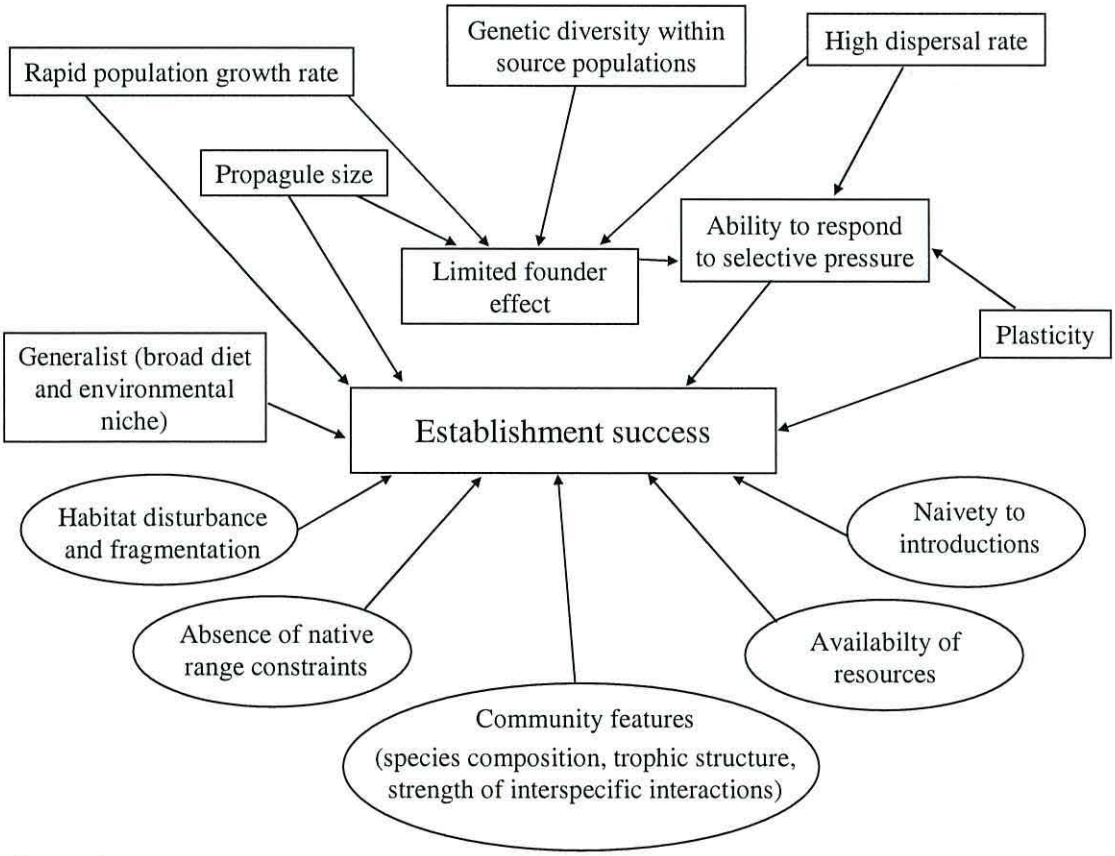

Figure 1.1.

Factors influencing establishment success. Boxes denote introduced population/species features, circles denote the novel environment's susceptibility.

Propagule pressure, the number of individuals introduced and the frequency of introduction events (Williamson 1996; Kolar & Lodge 2001), has long been linked to establishment success (Richter-Dyn & Goel 1972). The association is supported by theoretical predictions and empirical invasion studies (reviewed in Colautti *et al.* 2006). A large founding propagule can act as a demographic buffer against the mortality associated with the transportation to and encounter with novel environments and selective pressures. Large propagules are likely to contain a variety of alleles, some of which may be advantageous to the non-native in the new range. Subsequent introduction events are likely to increase establishment success by supplementing the demographic buffer against mortality. Multiple introductions are also an opportunity for the introduction of new haplotypes, which may alleviate genetic constraints to some extent (Kolar & Lodge 2001).

When a species encounters a novel environment, it may be confined to a small population and area (Fraser 1965), for example, the collared dove, *Streptopelia decaocto*, did not expand its range in Europe until the early 1900's, despite being introduced there 200 years previously (Hengeveld 1988). The functional interpretation of lag phases is difficult, but they may be a response to environmental uncertainty (Ewel 1986), or an artefact of a small founder population size (e.g. genetic constraints, Mack *et al.* 2000). Successful colonisation is most likely when introductions are mediated anthropogenically, since the direction and duration of transportation is often more sustained than in stochastically driven, naturally-mediated colonisations (Goel & Richter-Dyn 1974; Williamson 1996; Colautti *et al.* 2006).

Repeated, successful invasions by particular species in a variety of widely distributed geographical areas suggests that some species possess attributes which increase the likelihood of invasion success, and researchers have long attempted to identify these invasive characteristics (Henslow 1879; Baker & Stebbins 1965; Baker 1974). Generic, across-taxa traits that have been suggested include *r*-selected life-histories, rapid dispersal rates, a broad diet, high abundances in native ranges and the ability to function in a wide range of physical conditions (Ehrlich 1989). A high recruitment rate leads to rapid proliferation and is a common attribute of many conspicuous invasive species (Lodge 1993; Williamson 1996). For example, a single adult Zebra mussel (*Dreissena*

polymorpha), a European species introduced into the Ohio River Basin, can produce one million planktonic larvae per year, which, in turn reach sexual maturity within a year (Schloesser *et al.* 1996).

The ability to adapt and respond (phenotypically or genetically) to novel selective pressure is imperative for the continued success of introduced populations. Phenotypic plasticity is likely to be important for acclimatisation in the early establishment phase of introductions, prior to adaptive responses. The 'general purpose genotype'(Baker 1965) describes the important ability of colonising species to thrive in a range of environmental conditions through phenotypic or developmental plasticity. Plasticity is a key attribute which has enabled the widespread establishment of invasive salt cedar *Tamarix ramosissima* in cold climates of North America (Sexton *et al.* 2002). Invasion success may be facilitated by the presence of genetic variability in introduced populations upon which selection may act, a point which will be expanded upon later in this thesis.

The fate and impact of species introductions may be as much a result of the characteristics of the community as those of the introduced propagule (Lodge 1993). Few communities are entirely impenetrable (Usher 1986; Usher *et al.* 1998; Lodge 1993), but many differ vastly in their susceptibility to non-native introduction. Although it is difficult to generalise, the amount of available resources, absence of potential competitors and predators, and naivety, where native species have evolved in the absence of heavy competitors or predators, all contribute to the susceptibility of environments to invasion (Lee 2002), Fig 1.1. Consequently, communities that comprise these characteristics, such as island communities, are likely to be highly prehensible to invasion. Escape from biotic constraints, for example, native competitors, can release the non-native from hindering competitive interactions enabling fuller exploitation of previously shared resource bases. Invaders might escape from their enemies (predators, parasites) during the invasion (Torchin *et al.* 2003), described as the 'enemy release' hypothesis (Keane & Crawley 2002). This phenomenon is thought to be associated with invasion success, although the processes involved are still under some debate (Drake 2003, Coalutti *et al.* 2004). Establishment of the amphipod crustacean *Dikerogammarus villosus* in newly colonised areas (Torchin *et al.* 2003) has been linked to disassociation with *Microsporidium spp.* parasites which are present in the

native range (Wattier *et al.* 2007). Habitat disturbance and fragmentation generally promote the invasibility of communities (Elton 1958; Sakai *et al.* 2001) and may amplify natural levels of spatial heterogeneity, which presents a greater chance that an introduced species may find a suitable niche for colonisation and establishment. Human-induced (e.g. grazing, urbanisation) or natural (e.g. fire, flood) habitat disturbance may enable easier spread and penetration of novel environments (Williams 1969), if the native species can neither quickly acclimatise nor adapt to the disturbance.

The species composition of a community, the trophic structure and the strength of interspecific interactions between and within trophic levels are important interrelated elements that influence the susceptibility of a community to invasion (Sakai *et al.* 2001). Elton (1958) proposed that the species richness of a community is positively correlated with the 'biological resistance' it can provide against introduced non-natives. The proposition is linked to the vacant, or underutilised niche hypothesis (reviewed by Simberloff 1981), which suggests that communities that are relatively impoverished in numbers of native species are more susceptible to invasion because of reduced competition for available niches. Although these associations between species richness/ vacant niches and susceptibility to invasion may be oversimplifications for continental communities (Herbold & Moyle 1986), and should be interpreted with care, they may have more relevance in island communities (Mack *et al.* 2000). For example, many small oceanic islands are extremely susceptible to species introductions (Tsutsui *et al.* 2000), mainly a result of impoverished native communities. Large, established, species-saturated islands (for example, those of continental origin) tend to be more resistant to invasion, an effect generally attributed to the often higher levels of inter-specific competition and aggression present in multi-species, multi-level communities (Elton 1958; Lee 2002).

1.1.3 Ecological consequences of introductions

The ecological consequences of biological invasions have been cited as the second largest threat to global biodiversity, after habitat destruction (Walker & Steffen 1997). The main ecological and evolutionary consequences of introductions are summarised in Fig. 1.2. Interspecific ecological interactions may be direct (e.g. predation and competition) or indirect (e.g. habitat alteration and cascading trophic interactions),

affect both introduced and native species, and operate at a variety of levels from the individual gene through to multi-level community interactions. Potential effects on individuals include changes in growth and reproduction rates and morphological and behavioural adjustments (Parker *et al.* 1999). For example, the introduction of the Nile perch (*Lates niloticus*) in Lake Victoria has caused behavioural changes in native prey fishes (Lodge 1993). Hybridisation may introduce genes from natives to introduced species, conferring fitness traits advantageous in the novel environment (Ellstrand & Schierenbeck 2000). Population level effects include changes in the abundance, distribution and dynamics (age, sex structure and growth rate) of native populations. Invasive Zebra mussels (*Dreissena polymorpha*) in the upper-midwestern United States have increased water clarity by extensive phytoplankton filtering, causing native vascular aquatic plants to increase in abundance (Lodge 2001). Community and ecosystem level effects include changes in species richness, diversity and the intensity of interspecies relationships (Parker *et al.* 1999). For example, the natural vegetation of extensive open forest and savannah in Kentucky was replaced following European settlement and land clearing, by expansive fields of invasive bluegrass, *Poa pratensis* (Mack *et al.* 2000).

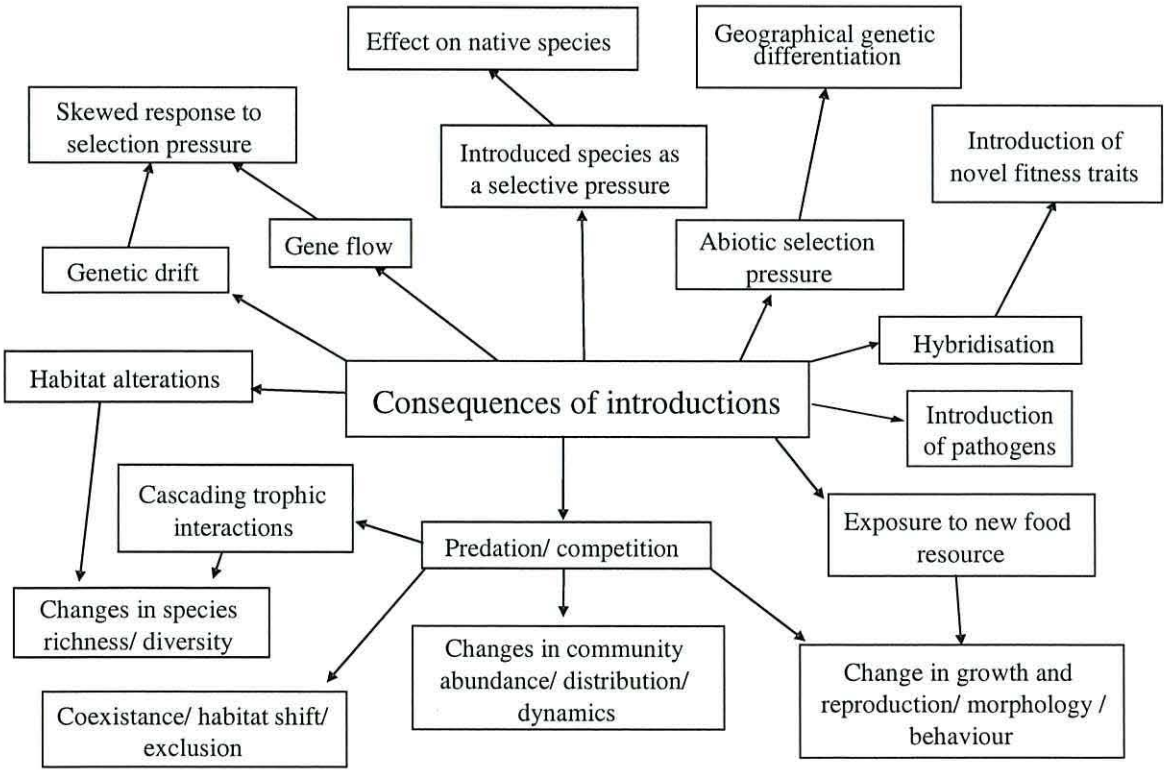

Figure 1.2 .

Ecological and evolutionary consequences of introductions.

For recent species introductions, the direct effects of competition and predation may be easier to detect, predict and observe than the indirect consequences that operate at multiple ecosystem trophic levels. These interspecific interactions have long been recognised as a significant force in shaping community structure and the geographic distributions of species. During invasions these forces are often intensified, and depending on direction and strength, can result in coexistence, niche space alterations, habitat shift or exclusion of either species (Shigesada & Kawasaki 1997). Almost by definition, introduction studies are most often concerned with situations where the introduced species is the dominant predator or competitor, and where interspecific interactions can accelerate range expansion and colonisation. Competition between invasive and native species for ecosystem resources can be subtle and complex (Williamson 1996), and although is usually notoriously difficult to prove, direct consequences of competition have been identified in some species introductions.

The more efficient foraging strategies of introduced gray squirrels (*Sciurus carolinensis)* have resulted in dramatically declining populations of native red squirrels (*Sciurus vulgaris)* in Britain (Williamson 1996). The introduction of pathogens can be detrimental to native populations. Another factor contributing to the decline of red squirells is the parapox virus, which can be fatal to red squirrels but does not result in death in gray squirrels. The spread of the amphibian fungal disease chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* has contributed to a worldwide decline in amphibian populations and spread of the virus has been blamed on international trade and movements of *Xenopus laevis* in the 1930's. The mass release of the European lady beetle (*Coccinella septempunctata*) for the control of Russian wheat aphid has, by out-competition, almost replaced some populations of the native American lady beetle *Adalia bipunctata* (Gordon & Vandenberg 1991; Elliott *et al.* 1996). Whilst predation on introduced species often serves to slow or limit spread and success, the negative effects of predation on natives may be severe, for example, predation by feral cats has been strongly implicated in the extinctions of six native Australian marsupials in the nineteenth century (Dickman 1996). Intra-guild predation, where a predator also competes with its prey for ecosystem resources has complex outcomes and special significance for species introductions. The predator can simultaneously benefit from energy gain and reduced competitive effects, and this

special case of predation has probably aided *Anolis sagrei* in replacing *A. carolinensis* in Florida and *A. conspersus* in Grand Cayman (Gerber & Echternacht 2000).

1.2 Adaptive evolution

1.2.1 Rates of Evolutionary change

Historically, evolution has been viewed as a slow process, the Darwinian perspective suggesting that gradual changes in organisms through the force of natural selection result in adaptation and accumulate to result in substantial change (Reznick & Ghalambor 2001). Darwin (1859) attributed the slowness of evolution to the weaker forces of natural selection compared to those of artificial selection. The fact that evolution had not been directly observed in nature at that point contributed to this conclusion. The earliest observations of evolution by natural selection (e.g. insecticide resistance and industrial melanism) were caused by anthropogenic influences, which were supposed to exert significantly stronger selective forces than natural coefficients of selection. However, since Endler's (1986) review, which suggested that coefficients of selection in nature could be high enough to be observable, growing numbers of empirical studies have indeed shown that evolutionary change is apparent in natural systems (Reznick *et al.* 1997; Thompson 1998; Hendry & Kinnison 1999). The scope of empirical studies has been widened since it has been acknowledged that adaptive change may be detected in timescales suitable for investigations. High rates of evolution facilitate evaluations of the sustained rate of change (Lynch & Lande 1993) and the association between natural selection and speciation (Schluter 1998). Moreover, empirical studies of adaptive evolution enable the linking of ecological conditions to the strength of natural selection and rates of evolutionary change. The formulation of such ecological associations facilitates predictions of the evolutionary outcomes of selection pressures. These predictions are especially valuable as it is increasingly acknowledged that anthropogenic influence has presented the Earth's biota with a multitude of novel environmental challenges on local, regional and global scales.

1.2.2 Evolution in introduced species

Species introductions present some of the best empirical examples for studying the interactive influences of ecological and evolutionary processes. Successful introductions are stochastic events, often involving small populations that survive significant transitions in environmental conditions. Frequently, establishment in new environments involves a complex and intense series of interactions between non-indigenous species, native species and the biophysical environment. The interplay between these factors together result in a set of novel selective pressures in a variety of directions and strengths, Fig 1.2. Thus, the successful establishment and proliferation of introduced species in their new ranges depends on appropriate responses to these selective pressures either by adaptation or plasticity. Phenotypic plasticity may permit immediate and reversible adjustment to environmental change, facilitating introduced species by increasing the range of phenotypic response to the novel environment, and enabling range expansion (Maron *et al.* 2004). However, although plasticity has been historically invoked for colonisation success, because it is non-heritable, it cannot fully account for the ability of introduced species to respond to selective pressure by local adaptation. Recent reviews suggest that invasion success may depend more on the ability to respond to natural selection, rather than phenotypic plasticity (Sakai *et al.* 2001).

Since species introductions frequently involve novel environmental conditions and short timescales, the opportunity for newly introduced species to undergo rapid adaptation is substantial. Empirical evidence from a number of recent studies has provided support for this (reviewed in Lambrinos 2004). Rapid evolutionary changes have been documented in morphological and behavioural traits of introduced species (reviewed in Cox 1999; Mooney & Cleland 2001; Reznick & Ghalambor 2001; Lee 2002). The invasive weed species, barnyard grass (*Echinochola crus-galli*) has evolved crop mimicry to evade eradication attempts with herbicidal agents (Barrett 1983). Recent work shows that the introduced Quebec population of this species (usually confined to lower latitudes due to a C4 photosynthetic system) evolved enhanced catalytic efficiency of some enzymes, enabling the invasion of cooler climates (Hakam & Simon 2000). Introduced populations of House finch (*Carpodacus mexicanus*) in eastern North America have evolved a complex system of migratory behaviours following their introduction from warmer western North America, probably southern California (Mundinger 1975; Able & Belthoff 1998).

Introduced species can themselves act as strong selective forces on species encountered in the newly occupied range (Reznick & Ghalambor 2001), a phenomenon which has largely been overlooked in the species introductions literature, although see Fisk *et al.* (2007). Recent experimental studies with natural populations have shown that resident *Anolis* lizards alter their habitat use in the presence of an introduced predator (Losos *et al.* 2004). Competition between the endemic anole *Anolis oculatus* and the recently introduced *Anolis cristatellus* from Puerto Rico has resulted in expulsion of the native species from the South-western coast of Dominica (pers. obs.). Moreover, environmental conditions and interspecific interactions may vary across the introduced habitat, introducing the opportunity for further, differential adaptive responses. Thus, empirical studies on evolutionary processes during species introductions offer a useful model for studying rapid adaptive responses to directional selection, and enable observations on the influence of ecological conditions on the rate of evolutionary change.

The rate, persistence and spread of genetically-based local adaptation caused by introductions is affected by interactions between genetic composition, dispersal and colonisation patterns, population dynamics and life-history (Lambrinos 2004). Demographic bottlenecks during introduction and genetic drift in small founder populations are expected to drastically reduce the levels of genetic diversity of invasive species, compared to those in their native range (the 'founder effect', Mayr 1942; 1954; 1963). However, because sufficient genetic variance is essential for adaptation (Fisher 1930; Reznick *et al.* 1997), the success of many invasive species contradicts the theoretical expectations. This apparent paradox, and indeed the genetic significance of founder effects (Nei *et al.* 1975), has remained highly debatable, and will be covered in a separate section of this thesis. Dispersal patterns can influence how quickly local adaptation can form (Lambrinos 2004), repeated colonisation can minimise and inflate genetic founder effects (Kolbe *et al.* 2004) and maintain a variety of phenotypes that increase the opportunity for adaptation (Maron *et al.* 2004). The opportunity for population growth enables sustained directional selection to positively influence introduced population survival and fitness. A number of studies have provided clear evidence for rapid evolutionary responses to local conditions in a number of species introductions (examples in Costa-Pierce 2003; Maron *et al.* 2004; Rasner *et al.* 2004; Yeh 2004; Ayllon *et al.* 2006).

Other evolutionary mechanisms apart from natural selection may be important in implementing changes in non-native species. Genetic drift may result in significant shifts in phenotype which affect the invasion success of a species (Templeton 1980; Carson & Templeton 1984). Genetic admixture amongst introduced individuals (for example, through multiple, sequential introductions) may provide a wide range of heritable phenotypes enabling response to local selection pressures (Sexton *et al.* 2002). Whilst high rates of gene flow may help distribute genetic diversity required for adaptive changes across species introduced ranges, it may simultaneously act to constrain adaptation to local conditions, and thus hinder population persistence. Conversely, too little migration may lead to extinction of newly introduced populations through demographic stochasticity, or the lack of genetic variation (Lenormand 2002; Nosil & Crespi 2004).

Hybridisation between native and introduced species is another mechanism which may facilitate adaptive evolution through gene flow. For example, hybridisation of *Spartina alterniflora* with the native *S. maritima* has resulted in a highly invasive hybrid, which has succeeded in displacement of the native species (Gray *et al.* 1991). Introduced species may sometimes radically alter the biotic and abiotic characteristics of their new habitats, thereby altering the strength and characteristics of selective pressures within them, and associated shifts in native species' responses to them. For example, large expanses of invasive grasses are known to increase the risk and frequency of fires, altering the community composition and nutrient cycling systems of these areas (reviewed by Brooks *et al.* 2004).

The range of selective forces that non-natives are exposed to in the novel environment is composed both of new selective agents and of the absence of previously significant selective agents in the home range. Biotic selection pressures that drive evolution include exposure to a new food resource, predator or coexisting competitor. Abiotic environmental pressure is also a significant selective force, and when varying across geographical gradients can promote genetically based differentiation amongst introduced populations (Sexton *et al.* 2002).

1.2.3 Geographic variation and adaptive evolution

Across many taxa, patterns of geographic variation can be used to elucidate evolutionary selective pressures to particular environmental conditions (Gould & Johnston 1972). Consequently, study of these patterns and their causes have provided important contributions to evolutionary theory (Endler 1977). Geographical variation is either the manifestation of plastic responses to the environment, or is adaptation based on genetically controlled traits. Patterns of variation based on heritable traits arise either through historical processes (such as dispersal and vicariance events) or natural selection (genetically-based adaptive responses to sustained directional selection, Thorpe 1987). The study of adaptation in introduced species is especially valuable because historical processes can often be discounted, thus the interactions between directional selection and phenotypic response are more evident.

The colonisation and dispersal across North America by House sparrows, *Passer domesticus* since 1851 demonstrates geographical trends in morphological responses to differing selection pressures (Lowther 1976). These selection pressures have been related to environmental factors, and the trends were found to be generally consistent with the ecogeographic rules of Bergmann and Allen (Johnston & Selander 1971). Although the influence of processes such as vicariance events can be removed for the majority of species introductions, the degree of genotypic admixture significantly affects the amount and type of variation present in introduced populations. Molecular analyses can help elucidate the role of multiple introduction and influence of genetic admixture.

Geographic variation can occur on a variety of levels, molecular (Avise *et al.* 1987), behavioural (Endler & Houde 1995), physiological (Clarke *et al.* 2000) and morphological (Mayr 1963). Morphological variation is the level at which most studies assess geographic variation because it is in many cases easiest to measure, expected to be highly heritable and easily related to differing selection pressures, due to its high functional significance (Soulé & Kerfoot 1972). Ecological processes that are likely to impart significant selective pressures include changes in available resources, biophysical environment, competitive interactions and mortality rates. Populations exposed to new climatic gradients can evolve associated clinal variation in traits important to survival in these climatic conditions.

Phenotypic plasticity is the other mechanism aside from natural selection, which results in phenotypic variation in response to ecological conditions. It describes the differences an individual can express in response to environmental pressures. Whilst phenotypic plasticity itself is non-genetic and therefore does not confer any evolutionary change, the inheritance of plasticity may enable a population to exploit an environment which would otherwise be inhabitable (Losos *et al.* 2000). Although plasticity is increasingly recognised as being able to contribute to adaptive evolutionary responses (Ghalambor *et al.* 2007), full elucidation of its role requires a separate experimental approach and this thesis will primarily focus on adaptive genetic responses.

1.2.4 Adaptive evolution in Caribbean anoles

The *Anolis* lizards of Central and Southern America and the Caribbean are an ideal genus for researchers to study evolutionary divergence and adaptive island radiations (Roughgarden & Pacala 1989; Losos 1992; Losos *et al.* 1997; Malhotra & Thorpe 1997a; Losos *et al.* 2000; Knox *et al.* 2001). Since the 1960's, the phylogeny of the *Anolis* has been analysed and reanalysed and several of the past species groupings have been hotly debated. For example, Etheridge (1959) separated *Anolis* into the 'beta' anoles (renamed as *Norops* by Guyer & Savage, 1986) and the 'alpha' group on the basis of distinguishing skeletal characters. Although *Norops* is a monophyletic group, it renders the 'alpha' anoles paraphyletic, a designation which is not supported by phylogenetic analyses (Jackman *et al.* 1997; Poe 2004; Nicholson *et al.* 2005). The *Norops* grouping is now considered be too problematic and has been widely rejected (Nicholson 2002). Recent molecular evidence has thus provided distinction to the karyotypic (Gorman *et al.* 1967; Gorman & Atkins 1969); protein (Gorman & Dessauer 1965, 1966; Gorman & Atkins 1969) and immunogenetic (Gorman *et al.* 1980; Shochat & Dessauer 1981) phylogenetic analyses of previous years.

The Caribbean anoles alone number over 150 species which descend from as few as two mainland species (Jackman *et al.* 1997). Adaptive diversification in these species is omnipresent, manifesting in three dimensions. First, Caribbean anoles present multiple instances of sympatric species: closely related species that occupy different niches within a habitat and have different morphologies, a phenomenon also noted by Darwin

(1859) when describing the finches of the Galapagos. Second, within islands, endemic Lesser Antillean anoles present highly structured intraspecific variation in a number of morphological traits. The extent of this difference is such that a suite of phenotypically variable 'ecotypes' has been recognized for the species that inhabit the larger, more ecologically diverse islands (Malhotra & Thorpe 1991b; Thorpe & Malhotra 1996). These traits, most often scalation, body dimensions and colour patterns, have been associated with considerable within-island environmental variation present on the volcanic Caribbean islands (Malhotra & Thorpe 1997b, Thorpe 2002). Third, repeatedly on the large islands of the Greater Antilles, entire anole communities appear to have converged, the same set of ecological specialists evolving on each island, apparently independently at least four times (Williams 1983). Repeated colonisation has been excluded as an explanation for the inter-island phenotypic convergence by phylogenetic comparisons of mitchondrial DNA sequences (Losos *et al.* 1998).

This degree of repeated community evolution is almost unrivalled, although communities in different areas often bear some resemblance to each other, the occurrence of communities composed of the same set of ecological specialists is very rare (Losos 1995). Caribbean islands are relatively impoverished in their fauna in comparison to nearby Central American regions, and this has enabled the anoles to radiate in an open niche to fill ecosystem gaps left vacant by potential competitor absentees and expand unrestricted by heavy predation levels (Losos 2001). This, coupled with the relatively similar environment found on each island, suggests that evolution may take the same course over again, as was the case in the radiation of cichlid fish of the Rift lakes in East Africa (reviewed in Stiassny & Meyer 1999). The existence of these highly convergent communities suggests that there is some strong deterministic process driving the anole's repetitive evolutionary path (Losos & Thorpe 2004). The often dramatic variation observed in anole phenotypic traits that varies with environment has been associated with functional advantages to different niches (Losos 1990; Losos *et al.* 1994). Parallel trends within and between Caribbean islands in *Anolis* phenoype-environment associations thus appear to have been the result of adaptive evolution.

1.2.5 Evidence for adaptive evolution

Strong associations between clines in species' phenotypic traits and environmental factors have previously been cited as robust evidence for population responses to selection. However, variation in phenotypic traits may primarily result from historical processes such as drift, rather than natural selection for varying environmental conditions. Thus, to determine the role of historical processes in geographically distributed phenotypic variation, genetic characterisation of populations is crucial. Following the recent accessibility of molecular tools, many investigations have attempted to provide genetic substantiation for natural selective processes acting on phenotypic traits, by accounting for historical processes (Ogden & Thorpe 2002; Costa-Pierce 2004). Furthermore, it is also possible that the observed phenotypic variation is based on plastic responses to environmental variability rather than genetically based selection. Estimates of trait heritability, common garden or reciprocal transplant natural selection experiments are required to exclude a major role for plasticity and prove that clinal phenotypic variation is predominantly under genetic control.

Within single species, Lesser Antillean anoles present highly structured phenotypic variation in a number of quantifiable traits (Malhotra & Thorpe 1997, Thorpe 2002). This variation has been associated with the substantial geographic variation in microclimate and vegetation that is common within the volcanic Caribbean islands (Malhotra & Thorpe 2000; Thorpe & Stenson 2003). The complex environmental heterogeneity has been attributed to the high elevation and highly structured topography of the younger, inner arc of the Lesser Antilles archipelago (Losos & Thorpe 2004). Mitochondrial DNA sequencing has enabled the substantiation of the primary role of natural selection over historical processes in the phenotypic variation apparent in Lesser Antillean anoles (Malhotra & Thorpe 1997b; Ogden & Thorpe 2002). Thus, ecological processes primarily drive the geographical patterns of intraspecific variation in *Anolis*.

To determine a genetic basis for phenotypically variable traits, manipulative rearing experiments are required. Without such studies, it cannot be assumed that the phenotypic variation is caused by plastic environmental responses. Indeed, for *Anolis*, recent studies have attributed seemingly adaptive phenotypic differences to plasticity (Losos *et al.* 2000), highlighting the importance of controlled experimental investigations. Common garden experiments have separated selective and plastic processes by measuring phenotypic differences between individuals from

environmentally different localities, hatched and reared in identical conditions (Thorpe *et al.* 2005). Common garden experiments may be supplemented with natural selection experiments, which test for the strength of directional selection by measuring mortality of individuals translocated to environmentally different conditions from those in which they were reared. Breeding experiments can provide heritability estimates for traits, for example, scale number (Calsbeek *et al.* 2006). Both independently and combined, these experiments have been used to demonstrate that geographically varying traits are under significant genetic control and are determined more by selective processes rather than plastic responses for some Lesser Antillean (Malhotra & Thorpe 1991a; Thorpe *et al.* 2005) and Greater Antillean anoles (Calsbeek *et al.* 2006).

1.3 Invasion genetics

1.3.1 The founder effect

Introduced species offer an ideal opportunity to study the interactions between genetic founder effects, novel selection pressures and demographic processes such as migration. Although past studies have often tended to focus on assessing the ecological impacts, the recent application of molecular techniques has enhanced understanding of the historical processes and evolutionary consequences of biological invasions (Lee 2002). Moreover, the limited genetic diversity associated with the typically small size of introduced populations is a problem also faced by many species which require conservation effort. Investigations into the genetic characteristics of species introductions therefore provide a crucial insight into understanding species' responses to a variety of demographic and evolutionary challenges, and consequently, have a wide scope outside the field of invasion biology.

The 'founder effect' was first described by Mayr (Mayr 1942; 1954; 1963) as the establishment of a new population by a small number of individuals derived from a larger source population. The founder population typically carries a small proportion of the genetic variation contained within the original source population (Gray 1986; Allendorf & Lunquist 2003). When all imported individuals originate from the same source population, the genetic founder effect is particularly severe. Genetic drift is the

principal mechanism by which the initial loss of genetic diversity via a sampling effect is exacerbated. The isolation of a small population initiates significant genetic drift because of the extreme reduction in genetic variation. Over generations, drift leads to random changes in allele frequency, and given time, results in the loss or homozygous fixation of alleles, thereby reducing genetic diversity. The sampling effect of founding population establishment, combined with genetic drift can result in a rapid alteration of the genetic characteristics (and the associated phenotypic characteristics) of the founder population, as compared to those of the source population. This effect may be of such intensity that founder events have been proposed as a mechanism of speciation in allopatrically divergent populations (Carson & Templeton 1984; Avise 1994), although there is little empirical evidence to support this idea (Coyne *et al.* 1997).

Inbreeding (the mating of close relatives) is an unavoidable consequence of small population size and if a founding population stays small for several generations, inbreeding can exacerbate the initial reductions in genetic diversity by exposing deleterious recessive alleles (Lande 1988; Leberg 1990; Mills & Smouse 1994; Frankham 1995; Sakai *et al.* 2001). This results in a significant decrease in the mean population fitness (due to the homozygous expression of these deleterious recessive traits), termed inbreeding depression (Falconer 1989). Inbreeding depression may limit population growth, increasing the probability that the introduced population fails to become invasive (Newman & Pilson 1997). The amount of genetic variation in a founding population is also dependent on reproductive ecology and life-history traits which may exacerbate or ameliorate founder effects. For example, asexual or self-fertilising species may escape inbreeding depression, since individuals are more likely to be dominant for advantageous traits, thus fewer deleterious recessive alleles are exposed in the F1 generation.

Genetic variation is the substrate with which introduced species can respond to the novel environment via selection. Introduction success may therefore be dependant on the amount of genetic variation available in the population to maximise short term fitness potential. However, species introductions typically involve small populations and are therefore subject to the founder effects described above. This contradicts the need for genetic variability for adaptive responses and combined with the deleterious effects of genetic drift and inbreeding on population fitness, suggests that founding

populations are at a fitness disadvantage in their new ranges. The widespread invasive success of many introduced species therefore demonstrates a common paradox in invasion biology: founder populations with typically low genetic diversity, low evolutionary potential and potentially low reproductive fitness can proliferate and become invasive (Sakai *et al.* 2001; Allendorf & Lunquist 2003; Vendramin *et al.* 2007). Indeed, introduced species in novel environments have some of the most rapid rates of adaptive evolution (Reznick & Ghalambor 2001). Additionally, species that possess low genetic diversity, as a consequence of a demographic bottleneck have been shown to demonstrate variation in adaptive traits and/ or a widespread distribution, for example, Mediterranean pine, *Pinus pinea* L., (Vendramin *et al.* 2007). Several ways for genetic variation to be maintained or increased following a demographic bottleneck have been suggested.

1.3.2 Surmounting the genetic paradox

The genetic paradox posed by the success of invasive species is an interesting conundrum for evolutionary biologists. The fact that the vast majority of introductions fail to establish, proliferate and become invasive may be, in part, an artefact of populations possessing limited genetic variability with which to respond to local, novel selective pressures. Accordingly, successful invasions should be instigated by founding populations that contain sufficient genetic variability for adaptive responses in the new range. Several mechanisms have been suggested that may increase the availability of genetic variation upon which selection can act. The introduction of multiple genotypes to introduced ranges can overcome initially low genetic variability. Lag phases may allow acclimatisation via phenotypic plasticity enabling introduced species to expand populations in the new range. Potential solutions to the genetic paradox, specifically the mechanisms by which genetic variation for selective responses can be increased in a small propagule without new mutation, are explored below.

Whilst the term genetic diversity is often used to indicate the potential for evolutionary adaptation, it is actually additive genetic variance (AGV) that provides the main substrate for natural selection (Lee 2002). AGV is an increase in genetic variation due to alleles with additive effects. An example of AGV is dominance deviation, which occurs when both alleles at a heterozygotic gene locus affect the genotype at that locus

(Frankham *et al.* 2001). The greater the genotypic difference between two homozygotes, the larger the increase in AGV. Sufficient AGV within a population is required for adaptation to environmental changes (Goodnight 1995), and much AGV is dependant upon heterozygosity. Therefore, in order that small founding populations with low heterozygosity can adapt, they must explore other options to increase AGV, without the generation of new mutations. Several studies have shown theoretical (Goodnight 1988; Willis & Orr 1993) or empirical (Bryant *et al.* 1986; Bryant & Meffert 1993; Cheverud *et al.* 1999) evidence that AGV can increase in small populations or in response to demographic bottlenecks. An important, non-mutational source of AGV is via the action of epistasis (Lee 2002).

Epistasis is the interaction of two or more alleles at different loci. This process can potentially increase AGV because the genotypic value of the interacting multiple loci deviates from the sum of genotypic values of loci considered singly. Epistasis is a ubiquitous phenomenon because of the intense interaction of genes and gene products affecting each other's expression. The greater the number of loci involved, the more potential AGV is available (Cheverud *et al.* 2001). Epistasis is created by natural (stabilising) selection, which produces a balanced, interacting system of genes working via coadaptation within their physiological genetic environment. This results in phenotypic uniformity across a population in stable environmental and demographic conditions at an adaptive value, despite underlying genetic diversity (Naciri-Graven & Goudet 2003). Evolutionary change may be difficult due to the intensity of this coadaptation, because alterations in one locus are likely to affect other loci in adverse ways, leading to evolutionary inertia. Although this inertia is adaptive when formed, it needs to be dismantled for further adaptation, and a founding event may provide this required disruption (Cheverud *et al.* 1999).

Epistatic gene interactions could contribute to AGV via natural selection, drift or both of these processes (Lee 2002). The genetic influence of epistasis is therefore amplified in small populations and under extended isolation (Goodnight 1987). Theoretical models indicate that novel and strong selective forces, together with population allele frequencies altered by drift, can disrupt the balance of coadapted gene complexes in founder populations, increasing AGV relative to ancestral populations (Goodnight 1987). Random genetic drift, an effect that is enhanced in small populations, occurs

when chance fluctuations in allele frequencies over multiple generations cause these allele frequencies to drift, resulting in the loss of genetic diversity and the fixation of alleles (Cheverud *et al.* 1999). Although genetic drift is a process that generally reduces diversity, there is theoretical evidence to suggest it has a role in increasing AGV (Cheverud *et al.* 1999).

The brief, intense periods of random genetic drift which frequent small founder populations can disrupt coadapted gene complexes, freeing the loci from each other and the multiple adaptive peaks that they combine to form, and promoting genetic change for adaptation (Goodnight 1988; Willis & Orr, 1993; Cheverud *et al.* 1999). Genetic drift may result in random linkage disequilibrium among unlinked loci. This can allow new, possibly favourable gene complexes to appear (Wright 1977; Mayr 1942, 1954, 1963; Carson 1975), resulting in a 'genetic revolution', or a shift towards new and higher adaptive peaks (Mayr 1963). Thus, natural selection is able to act on a new system of coadapted genes working via epistasis (Cheverud & Routman 1995). It is well known that many alleles affecting fitness components and other quantitative traits are partially recessive (Simmons & Crow 1977). The exposure of previously rare, recessive homozygotic alleles to selection by drift drives allele frequencies to extreme values and creating associated disproportionately large increases in AGV (Willis & Orr 1993) when the frequency of recessive alleles is relatively small (Cheverud *et al.* 1999).

Naciri-Graven & Goudet (2003) found that as the number of loci involved in epistatic interactions increased, AGV increased, suggesting that epistasis, rather than dominance deviation plays a significant role in the increase of AGV following bottlenecks. The relative contributions of epistasis and dominance deviation to the potential increase in AGV in founder populations may be unclear and are likely to vary on a case-by-case basis. However, it is evident that increases in AGV are possible and can result in the availability of genetic variance upon which selection can act. The effect of increasing AGV may only be temporary but it may be sufficient for response to a new environment (Cheverud *et al.* 1999).

The genetic founder effect can be offset by the subsequent, additional introduction of multiple genotypes. This provides the opportunity for gene flow, recombination and reassortment of genotypes, which in turn, increases genetic variation. A growing

number of studies highlight the importance of multiple genotype introductions in maintaining genetic variation in invasive populations (Genton *et al.* 2005; Therriault *et al.* 2005; Dlugosch & Parker 2007). High genetic variation both within and among populations of the invasive red swamp crayfish *Procambarus clarkii* can be explained by multiple source introductions (Barbaresi *et al.* 2003). Kolbe *et al.* (2004) showed multiple introductions of *Anolis sagrei* into Florida from the native Cuba and gene flow within Florida resulted in some Floridian populations containing higher genetic variation than in some native Cuban populations.

An often observed phenomenon in founder populations, and indeed in many populations experiencing a change in environmental conditions, is a lag period. The lag time occurs between initial colonisation and the onset of rapid population growth and range expansion (Mack 1985). At the beginning of this phase, phenotypic plasticity likely enables introduced populations to tolerate new local conditions and acclimatise to them (Sultan 1987). Following this acclimatisation, the lag time may be a requirement for the accumulation of AGV (Lee 2002). Consequently, during the lag phase, introduced populations may be able to evolve adaptive traits in the novel environment, enabling the population growth and range expansion that follows.

An interesting exception to the genetic paradox is the example of invasive Argentine ant, *Linepithema humile*, whose reduced genetic variation is associated with a reduction in intraspecific aggression, a trait in this species which leads to ecological success in this unicolonial species (Tsutsui *et al.* 2000). Further studies by Tsutsui *et al.* (2003) suggest that the ant has seemingly evolved to reduce genetic variation in invading populations, by selection against individuals from genetically diverse groups. This selection, when combined with founder effects is likely to both prevent the introduction of new genetic material from the native range, and reduce genetic variation within the introduced range (Tsutsui *et al.* 2003).

Genetic founder effects have been documented in a number of empirical examples (Baker & Moeed 1987; Hawley *et al.* 2006). However, an increasing number of studies from a diverse range of taxa in varying ecological circumstances fail to detect a loss of genetic diversity associated with invasion. Reviews of the literature suggest a great variation in the genetic characteristics of invasive species; some invasive populations

acquire significant genetic variability, others do not (Roman & Darling 2007). Much of this variety is probably due to differences in life-history characteristics, and the individuality of each introduction event. Although some genetic diversity is lost following founding events (most noticeably in allelic richness and heterozygosity), in many cases, gene flow acts to ameliorate this loss (Dlugosch & Parker 2008). Anthropogenic influences can often increase the amount of gene flow by multiple introductions via commercial transportation routes. There is mounting evidence from empirical studies that the subsequent introduction of multiple genotypes is a component of many successful introduction events (Ellstrand & Schierenbeck 2000; Allendorf & Lundquist 2003; Facon *et al.* 2003; Kolbe *et al.* 2004; Bossdorf *et al.* 2005; Frankham 2005; Novak & Mack 2005). Although there may be simple demographic advantages to multiple successive introduction events, it is likely that they also play a key role in the provision of genetic variability important for the invasive success associated with adaptive responses.

1.3.3 Phylogenetic reconstruction

Molecular phylogenetic methods reconstruct inter- and intra- specific relationships by assessing DNA base differences. From molecular phylogenies, inferences can be made about the historical processes (e.g. geological, anthropogenic effects) underlying the relationships between taxa (e.g. Canestrelli *et al.* 2006), as well as estimates of demographic parameters (Emerson *et al.* 2001). Sequence data may also be used to infer demographic parameters of past population histories, and methods commonly used to infer these parameters are often underpinned by coalescent theory (Kingman 1982). Coalescent theory states that any two haplotypes will have shared a common ancestor at some point in evolutionary history. Population size and mutation rate are determinants of the point at which the coalescent event occurred. Since small populations have a higher probability that haplotypes contained within them coalesce more recently than do larger populations (due to the stronger effect of genetic drift in the former) this understanding may be used to infer changes in population size over time. Coalescent-based methods use the distribution of divergence times among haplotypes to estimate effective population size. For more recent events in population dynamics (for example, the demographic histories of recently introduced populations), coalescent-based methods are largely inappropriate because the timescales involved (within decades or

centuries) are such that genetic drift is negligible. Furthermore, estimations based on coalescent theory may be unreliable unless multiple loci are used, because estimates of parameters have very wide confidence limits.

The inclusion of phylogenetic data has enabled the contextualisation of innumerable studies and illuminated many aspects of ecological and evolutionary biology; for example, the origin of West Indian anoline fauna (Hass *et al.* 1993). Mitochondrial DNA (mtDNA) is widely used for phylogenetic reconstruction because the mutational rate is generally higher compared to nuclear DNA (Taberlet 1996), thereby enabling a finer-scale resolution of relationships. This is due to a less sophisticated DNA repair mechanism and a lower effective population size for maternally inherited, haploid mtDNA compared to biparentally inherited, diploid nuclear DNA (Wilson *et al.* 1985). Furthermore, because the evolutionary rate differs for various mtDNA genes (Taberlet 1996), the gene with the appropriate level of variability for the phylogenetic level of interest can be selected. Due to the uniparental (usually maternal) pattern of inheritance, the pattern of mutation used for phylogenetic reconstruction is unperturbed by recombination (Avise & Vrijenhoek 1987; Avise 1994). In a few cases, the uniparental inheritance of mtDNA may provide a distorted evolutionary history; for example, for species with sex-biased dispersal (Taberlet 1996). Since the entire mitochondrial genome is inherited as one linked unit, it acts as a single locus with multiple alleles, and thus may be sensitive to selective sweeps. Tests of neutrality (e.g. McDonald & Kreitman 1991) should therefore be applied to mtDNA data prior to analysis to determine that the locus of interest is not likely to be under a significant selective force.

Across many taxa, phylogenetic reconstructions based on mtDNA sequence data remain the most popular, practical and successful tool for the resolution of phylogenies on a wide variety of taxonomic levels. The most frequently used analytical methods for the reconstruction of phylogenies are based principally on parsimony, likelihood or Bayesian approaches. Parsimony methods assume the minimum possible number of mutational character state changes between haplotypes. Haplotype networks based on parsimony (Templeton *et al.* 1992) are a useful method to visualise the mutational differences between haplotypes, number of individuals containing each haplotype, and importantly, their geographic distribution. From haplotype networks, inferences can be made on population expansion ("star-like" patterns), abundance and distribution of

haplotypes over geographical space. The phylogenetic tree produced using Maximum Parsimony (MP) methods is the tree that requires the least number of evolutionary changes (Page & Holmes 1998). This method assumes that evolution occurs by the fewest character state changes possible, and applies a constant rate of nucleotide substitution to the entire data set. However, this assumption is an oversimplification of nucleotide substitution and can severely affect the reliability of MP returned trees. For example, as nucleotide sequences accumulate substitutions over time, there is an increasing probability that some of these substitutions will occur at the same sites and can lead to a particular site becoming "saturated" with substitutions and becoming phylogenetically uninformative. Parsimony erroneously interprets similarities in bases at highly variable sites as convergent events and unreliable trees can be returned due to "long branch attraction".

These problems can be minimised by using likelihood-based (Maximum Likelihood or Bayesian inference) methods which incorporate a given model of nucleotide evolution (or substitutional model) which is estimated to fit the data set best. Such models make explicit assumptions, or account for various parameters that can affect the pattern of nucleotide differentiation seen in sequence data (Swofford *et al.* 1996). Methods which are based on these assumptions can return a more reliable tree, provided the substitutional model chosen represents the data correctly. These models have themselves evolved (in pace with advances in computing technology) to become increasingly complex, incorporating increasing parameters to explain nucleotide evolution. The starting point was the Jukes-Cantor model which contains a correction for saturation. The Kimura 2-Parameter assumes different rates of transition and transversion substitution events. The more complex substitutional models such as General Time Reversible (GTR) incorporate different frequencies of nucleotides, allow for the reversal of character states and incorporate differential rates of substitution amongst OTUs, calculated from the sequence data (Hills *et al.* 1996; Li 1997). The most appropriate model of evolution (and its parameters) to fit a data set may be selected by using a program such as MODELTEST (Posada & Crandall 1998), which sets evolutionary model parameters by which to generate a tree according to different evolutionary models, and uses other criteria (e.g. Akaike Information Criterion or likelihood ratio tests) to determine which model produces the most reliable tree.

Maximum likelihood (ML) methods use a likelihood optimality criterion that is based on a substitutional model with fixed parameters estimated to be the most suitable for the data set. From a starting tree (for example, a tree produced by simple neighbour-joining algorithm) a likelihood score is calculated (i.e. the probability of the observed data given the chosen evolutionary model). The branches of this tree are swapped to create a new topology, if the new tree has a higher likelihood value than the previous tree, the new tree is saved (only one tree is saved at any time). Following a heuristic search (which continues for a user-defined number of generations to minimise the potential of arriving at a local, not global, optimum), a single tree with the highest likelihood score is retained.

Bootstrapping methods are commonly used to assess confidence in the returned MP and ML trees (Felsenstein 1985). Usually, non-parametric bootstrapping methods are used, which generate replicate character matrices (pseudoreplicate bootstrap data sets) by randomly resampling from the original data set. For each pseudoreplicate data set, a tree is constructed, and from a (user-defined) number of the replicate data sets, a corresponding number of trees are produced. The proportion of these trees in which a particular node is supported (i.e. a clade repeatedly occurs) is presented as a percentage bootstrap value. The ML optimality method searches for the tree that produces the observed data with the highest likelihood, and is computationally intensive and time-consuming, because likelihood scores take longer to compute when increasingly complex evolutionary models are used, due to increased model parameters to calculate for each tree. Furthermore, usually, the more OTU's that are included in the data set (and the longer the sequence that is analysed), the slower the tree search becomes, because the possible space in which the method has to search increases exponentially.

Recently, Bayesian inference based methods have been used in phylogenetic reconstruction. This method produces trees with likelihood scores based on a substitutional model, whilst incorporating prior information about the probability of the topology being correct. Bayesian phylogenetic methods search for topologies which maximise the posterior probability, which is proportional to the product of the prior probability (the probability the topology is correct before the data is seen) and the likelihood (likelihood scores based on a substitutional model). A key Bayesian concept is that since the parameter (e.g. tree topology) is unknown, it is sensible to specify a

range or "probability distribution" to describe its possible values (Yang 2006). Thus, whilst ML will select the tree (from the trees produced from a heuristic search), that is most likely to be correct given the observed data, Bayesian inference generates a set of trees which are roughly equally likely to have generated the observed data. Simply put, ML methods assess the probability of a tree being correct given the data, whilst Bayesian methods assess the probability that the data has been generated by a particular tree. Another difference between ML and the Bayesian approach is that instead of the model parameters being fixed, they are free to vary to best fit the data for each tree.

Bayesian inference methods take advantage of a conditioned search algorithm called MCMC (Monte Carlo Markov Chain) to reduce the time cost of searching, a major factor in the increased popularity of Bayesian inference. This algorithm involves randomising both the model parameters and the branch topology to generate trees, then calculating the likelihood score for each tree. After a certain number of iterations (the "burn-in" period) MCMC searches iterate towards tree topologies that have similar, maximal likelihoods (a probability distribution) because the topologies of trees are dependant on the topologies of the immediately preceding tree. In other words, the MCMC searching algorithm visits the regions of parameter space in proportion to their probabilities. A posterior probability distribution of trees is created, a subset of which are sampled and saved, and a consensus tree of all these saved trees is created, with posterior probabilities at nodes. A posterior probability value at any particular node is the percentage of trees from the posterior probability distribution that contain the clade. Advantages of Bayesian inference over ML include the fact that considerable time is saved using MCMC because the likelihood is not maximised. Instead of a single phylogeny, a quantification of the strength of the phylogenetic signal in the data is returned, which provides a better representation of the expected phylogenetic relationship. The inclusion of prior probability is a key difference between Bayesian and ML method and is also the basis of its main criticism. This is due to the fact that the most reasonable prior distribution to include may be unclear or subjective.

Relationships between phylogeny and geography are referred to as phylogeographic patterns. The recognition of historical influences on patterns of genetic differentiation are an important consideration for biodiversity analysis and community ecology (Losos 1996). These historical patterns may in turn generate geographical patterns of

genetically controlled phenotypic traits. Phylogeographic investigations can reveal congruence between ecological and evolutionary processes, morphological differentiation and sequence divergence (for example, within-island microgeographic variation in *Gallotia galloti* in Tenerife, Thorpe *et al.* 1994; Thorpe *et al.* 1995). Phylogenetic analysis has contributed significantly to the understanding of range expansions, for example, those following the ice ages, identifying both geographical areas of refugia and corridors of spread (Hewitt 2000).

Phylogeographic analysis has been used to reveal colonisation and dispersal patterns in the widespread and speciose Caribbean *Anolis*. Data from a number of studies (reviewed in Jackman *et al.* 1997) indicate that the pattern of colonisation by *Anolis* lizards in the Caribbean followed two pathways. Within the Lesser Antilles, the *roquet* group colonised the southern islands, and the northern islands were colonised from north to south by the *bimaculatus* group (Stenson *et al.* 2004). Aside from its key role in elucidating historical distributional patterns, phylogenetics has also been particularly valuable in separating the roles of natural selection (environmental conditions) and allopatric differentiation (dispersal history) in creating patterns of intraspecific morphological variation in island *Anolis*. Mitochondrial DNA phylogenies for *A. oculatus* on Dominica (Malhotra & Thorpe 2000) and *A. roquet* on Martinique (Thorpe & Stenson 2003) indicate that geographical patterns of morphological difference are not necessarily associated with lineage.

The successful application of phylogenetics in revealing past patterns of colonisation suggests that similar evaluations could shed light on more recent histories, specifically, those of invasive and recently introduced species. Phylogenetic evaluation of molecular sequence data from native and introduced ranges of invasive species can assign individuals from introduced ranges to their most likely population of origin in source regions, and deduce temporal and spatial colonisation patterns. Many studies have successfully applied phylogenetic techniques as a tool for identifying source populations (Le Page *et al.* 2000; Grapputo *et al.* 2005; Lindholm *et al.* 2005). Although much variation may be lost during a demographic bottleneck (Villablanca *et al.* 1998), mtDNA data can provide information about molecular variation in cases where there has been a multiple introduction history (Kolbe *et al.* 2004; Grapputo *et al.* 2005). MtDNA may have a limited use in the assessment of within-population genetic

variability in both source and introduced populations and the associated detection of genetic founder effects. As more fine-scale markers have become widely popularised, their application in empirical studies has provided a more appropriate population genetic tool considering the short timescales common to many species introductions (Goodman *et al.* 2001; Tsutsui *et al.* 2001; Waldick *et al.* 2002).

1.3.4 Molecular markers for population genetics

The study of population genetic variation requires a sensitive, reliable and repeatable method to elucidate genetic differentiation between and within demes. Molecular markers are frequently used as an efficient way of assessing genetic variation by using limited numbers of loci, upon which a scale of variation can be constructed. Over the past 20 years, a variety of techniques for measuring DNA base variation using molecular markers have been developed. Many of these techniques fall outside the scope of this study, for further details see Frankham *et al.*(2002) and Avise (1994). The application of molecular techniques to empirical genetic data allows direct comparison of the relative levels of genetic differentiation at a range of taxonomic levels. This raw data can be subsequently analysed using an expanding plethora of analysis software to make inferences on a range of demographic, stochasitic, and ecological processes affecting patterns of population genetic variability.

Molecular markers have proved particularly useful for measuring local gene flow (e.g. AFLPs in the Martinique anole, *Anolis roquet*, Ogden *et al.* 2002) and dispersal within species (e.g. microsatellites used to reveal migration patterns in sea trout *Salmo trutta*, Ruzzante *et al.* 2004). They have been used to measure effective population size through the between-generation comparison of allele frequencies (e.g. the assessment of genetic variability within fish hatchery stocks, Allendorf & Ryman 1987). Molecular markers have also been widely used to detect past demographic bottlenecks though allele frequency distortions (e.g. microsatellites in newt (*Triturus* sp.) populations, Jehle & Arntzen 2002). Although molecular markers are most often used to reveal intra-specific patterns, some inter-specific discrimination is possible (e.g. genetic distinction of morphologically and geographically similar Eurasian voles, *Microtus spp.* using SNP data, Belfiore *et al.* 2003).

Understanding the utility and limitations of the available molecular markers is important when choosing the most appropriate technique for an investigation. Ideally development of markers should be cost and time efficient, provide multiple, independent markers, use small (and even partially degraded) tissue samples, be reliable and replicable, and require no prior information about an organism's genome (Mueller & Wolfenbarger 1999). The resulting markers should have a known mutational pattern, codominance for high confidence levels in the results, and an appropriate degree of variability for the scale being considered.

Popular types of molecular marker include microsatellites, minisatellites, Amplified Fragment Length Polymorphisms (AFLPs), Random Amplified Polymorphic DNA (RAPDs) and Restriction Fragment Length Polymorphisms (RFLPs). These methods have enjoyed varying levels of popularity following their development. RAPDs and RFLPs (of mitochondrial DNA) are less frequently used now because of their lower sensitivity and the widespread use of the more recently developed markers, for example, microsatellites and Single Nucleotide Polymorphisms (SNPs). Microsatellites possess a number of advantages over other molecular markers and have been used in a variety of studies, especially investigations of population structure and gene flow (Ogden & Thorpe 2002) and in assessing the extent of genetic founder effects (Beaumont & Bruford 1999). An overview of the background, application and analysis of microsatellites is described below.

Microsatellites are stretches of DNA within the nuclear genome that consist of tandem repeats of a simple sequence of nucleotides, each repeated sequence typically ranging between two and seven base pairs long (Hancock 1999). The number of microsatellite repeats is often highly variable and it is the variation in the number of repeats which is applied to assess genetic variability (Tautz 1989). The source of this variation is predominantly through 'slippage' during DNA replication, often resulting in a different number of repeats than the template (Amos 1999), if the slippage error escapes DNA repair mechanisms (Eisen 1999). Because the most common changes are in a single repeat unit and thus approximate to a stepwise mutation process (Estoup & Cornuet 1999), the stepwise mutation model (SMM, Ohta & Kimura 1973) has been used to explain polymorphism in microsatellites. However, because mutations may also lead to large increases in microsatellite repeat number in single steps, the two-phase model

(TPM) has also been advocated as a mode of mutation (Di Rienzo *et al.* 1994). Many factors affect the pattern of mutation in microsatellites, and the nearest approximation to the true mutational mode for any particular locus may lie between the two models. Thus, a model which incorporates a mixture of the models at different proportions may be the most appropriate.

Microsatellites are found preferentially in non-coding regions of the genome, which would suggest they have a minimal role in genetic function (Schlotterer 2000). Because microsatellites have discrete loci, co-dominant alleles, and are hypervariable (Mueller & Wolfenbarger 1999), they have recently been the most popular markers for intraspecific genetic studies (Goldstein & Schlotterer 1999). Genetic variation in microsatellites is resolved by the design of specific primers that anneal to either side of a microsatellite locus, which is amplified using polymerase chain reaction (PCR). The number of repeats at a given locus is easily revealed by fragment length variation using gel electrophoresis on acrylamide gel, or using a capillary sequencer.

The main disadvantage of microsatellites is that most loci are species-specific and thus require individual development taking considerable molecular skills, time and effort, although in some cases markers from closely related species may be used. It can be difficult to obtain microsatellite sequences from some species, probably due to variation in the amount of microsatellite sequences present in particular species, for example, the reported low frequencies of microsatellites isolated from *Lepidoptera*, Scott *et al.* (2004). Slippage and single base shifts during PCR can result in sizing problems (Beaumont & Bruford 1999). Most microsatellites have simply been amplified by PCR and used to investigate variations in length, and thus, there may be much hidden genetic variation within the sequences, caused by interruptions (Weber 1990) or compound microsatellites made up of several tandem repeats. The simple examination of fragment length may give a distorted estimation of genetic variability.

Despite these caveats, microsatellites remain an invaluable tool for population geneticists and have been used to examine hybridisation, population structure, phylogeography, population bottlenecks and inbreeding in a number of species (reviewed in Goldstein & Schlotterer 1999). Comparisons of variation in microsatellite allele frequencies showed that reductions in gene flow were concordant with divergent

selection for habitat type for *Anolis roquet* in Martinique (Ogden & Thorpe 2002), supporting the view of ecological speciation due to divergent natural selection. Many studies have used microsatellites to illustrate large reductions in genetic diversity during invasion events in a variety of taxa (Meunier *et al.* 2001; Durka *et al.* 2005; Lindholm *et al.* 2005).

Estimations of population parameters to reveal structure and differentiation (for example, reduced genetic diversity within introduced populations) using microsatellite data require both within and among population approaches. The identification of reduced genetic diversity within populations relies on the expectation that rare alleles are lost rapidly during a demographic bottleneck. This rare allele deficiency is compared to other allele parameters to detect a significant loss of rare alleles relative to pre-bottleneck proportions (Maruyama & Fueret 1985; Luikart & Cornuet 1998). Comparisons among populations and sub-populations can reveal differentiation based on geographical or historical separation, for example between source populations and introduced populations. A commonly used statistic in microsatellite analysis is F_{ST} (a division of Wright's *F*-statistics, Wright 1951) that compares subpopulation allelic diversity to total population allelic diversity. It is frequently analysed using Weir and Cockerham's (1984) method based on analysis of variance of allele frequencies for multiple alleles and loci. Other comparative statistics which are useful at the among population level include allelic richness, H_E expected heterozygosity (Nei 1973) and H_O observed heterozygosity and Nei's F_{IS}, the divergence of observed heterozygosity from expected heterozygosity per locus.

Although widely used, *F*-statistics may not be appropriate for the analysis of some microsatellite data. *F*-statistics assume approximation of microsatellite mutation to the Infinite Allele Model (IAM), in which each mutation event creates a new allele at a constant rate of mutation (Kimura & Crow 1964). This model is unlikely to be valid for the majority of microsatellites which may conform better to other mutational models (SMM or TPM) models. However, because computational simulations suggest that for less than 20 loci, *F*-statistics provide more accurate estimates than alternative methods (e.g. R_{ST}, Slatkin 1995), F_{ST} based estimates are deemed appropriate for the microsatellite analysis contained within this study. Bayesian methods cluster individuals

into population groupings whilst minimising Hardy-Weinberg disequilibrium between loci within groups (e.g. Cornuet & Luikart 1996; Pritchard *et al.* 2000). An advantage of Bayesian-based methods is that because no a priori assumptions about population groupings are made, they are sensitive to fine-scale genetic differentiation. Recently, Bayesian methods based on coalescent theory (Kingman 1982), using MCMC sampling methods, have been applied to microsatellite data (e.g. MIGRATE, Beerli & Felsenstein 2001; LAMARC, Kuhner & Smith 2007). However, these coalescent-based methods require extensive sampling of populations to avoid spurious estimates and apply the SMM model (Ohta & Kimura 1973) which is an over-simplistic approximation of microsatellite mutation.

1.4 The Caribbean

1.4.1 Islands and the Caribbean archipelago

Island communities have been at the forefront of evolutionary studies since the discovery of the process of natural selection (Darwin 1859, Wallace 1880). During this time, islands have long been recognised as valuable models for the study of faunal build up and competition. Island archipelagos offer ideal natural laboratories for evolutionary studies due to their small scale, isolation and endemicity. Macro-evolutionary studies based on island communities have provided empirical examples for the processes of colonisation, radiation, and dispersal (Grant 1956; MacArthur & Wilson 1967; Whittaker 1998). Modern phylogenetics has enabled the reconstruction of species relationships and have provided evidence for inter-island species divergence and evolutionary relationships (Carson & Kaneshiro 1976; Hass 1996; Jackman *et al.* 1999; Creer *et al.* 2001; Emerson 2002). Intra-specifically, studies within islands have provided important examples of species that have undergone evolutionary processes under restricted habitat and niche space. On islands that exhibit high ecological diversity and low species diversity, species are prone to phenotypic variation, population differentiation and natural selection. Thus, geographically and environmentally diverse islands provide opportunities to study these processes, often under short timescales and small geographical distances (Knights 1979; Brown *et al.* 1993; Malhotra & Thorpe 1994; Grant 1998).

The Caribbean archipelago is an older and more numerous island system than the other well-studied Galapagos and Hawaiian islands. The Caribbean chain can be split into three island groups: the Greater Antilles, Lesser Antilles, and the Bahamas. The Greater Antilles comprise Puerto Rico and the Virgin Islands, Jamaica, Hispaniola and Cuba, Fig. 1.3. Although there is evidence of volcanism in the Greater Antilles, the islands are composed largely of a partially submerged continental mountain range of Creatceous and Early Tertiary age, which form the precipitous backbone of each of the large islands. Sedimentary rocks, which are mostly limestones of Oligocene to Pliocene age, overlie the volcanic rock. These underlying faulted and folded volcanic and sedimentary rocks have been interrupted by local intrusions of igneous rocks.

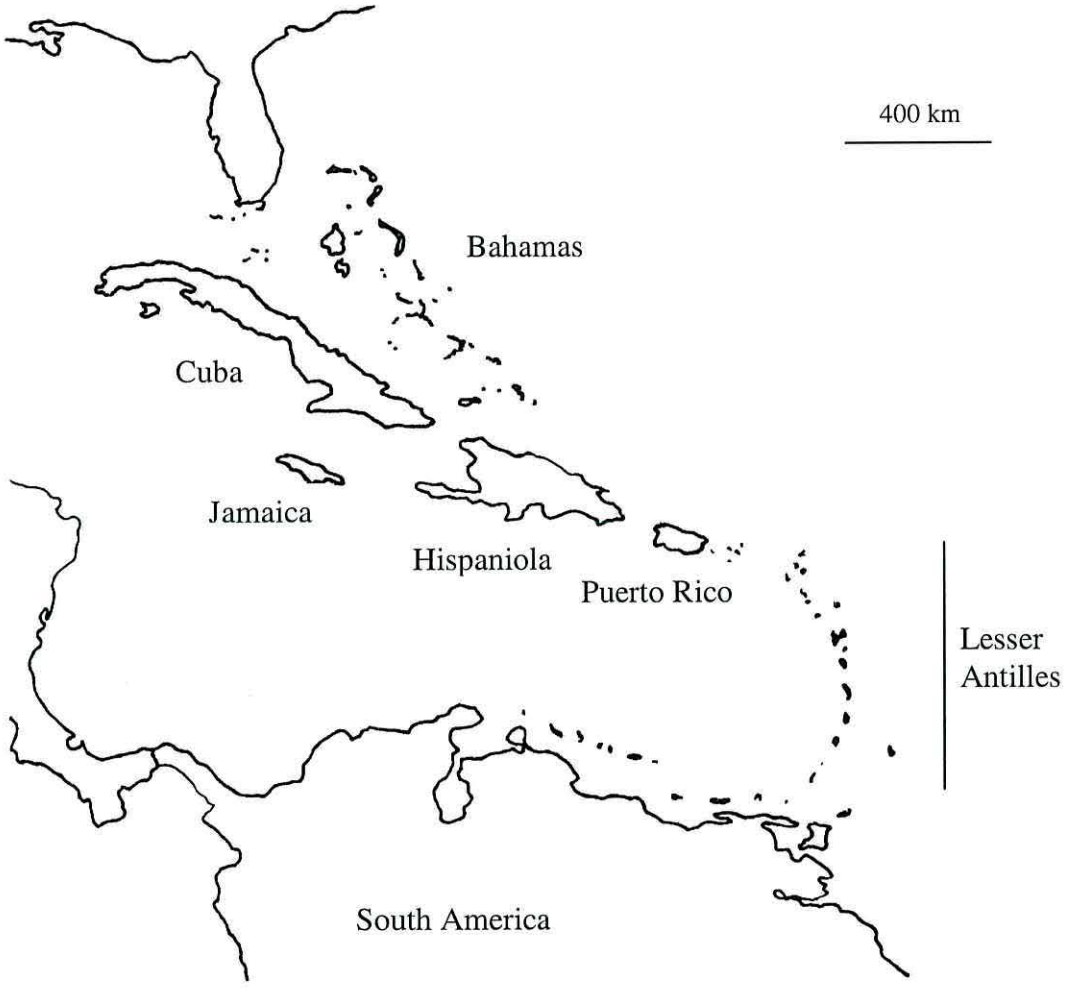

Figure 1.3
Greater and Lesser Antilles.

The Lesser Antilles comprise around 20 main islands and many smaller islets in the eastern Caribbean, Fig. 1.3. They range from Sombrero in the north, to Grenada in the south, adjacent to the continental island of Trinidad. The Lesser Antilles comprise two volcanically derived island arcs (Martin-Kaye 1969). The outer arc is the older of the two, dating to around the Eocene or early Miocene, and is characterised by low-lying islands formed by extensive, repetitive capping and erosion of limestone. The islands of this outer arc are volcanically extinct. The younger, inner arc dates from the late Miocene and is dominated by topographically complex islands showing evidence of more recent volcanic activity. The Lesser Antillean islands of Martinique and St. Lucia are exceptions, comprising land which originates from both outer and inner arcs. The Bahamas are carbonate platforms exposed following sea level drop in the Pleistocene and accumulated during subsequent rise in sea level during the Holocene. This thesis is concerned with species endemic to or inhabiting the Lesser Antillean islands of Dominica and St. Vincent and the Greater Antillean islands of Puerto Rico and Cuba. The climate, geology and vegetation of these regions and islands are summarised below.

1.4.2 Lesser Antilles and Greater Antilles : climate, geology and vegetation

The latitudinal position of the Lesser Antilles (and Greater Antilles) between the tropic of cancer and the equator confer a tropical climate, which varies between islands due to the combined effect of tradewinds and island topography. The description below relates to the inner arc Lesser Antilles. The rainy season extends from around June until December, during which time, tropical storms and hurricanes may occur. Average temperatures generally vary between 24°C in January and 30°C in June, although on the highest and most exposed peaks temperatures can be as low as 13°C. The prevailing north easterly trade winds induce orographic cloud formation and result in precipitation over high elevations in the interior of the islands.

The volcanic, late Miocene age bedrock of the inner arc Lesser Antilles is interspersed with layers of limestone deposits, and soils are generally clay-based. Tropical forest in this region is typically high in species richness and endemics. Mature moist forest is often dominated by an association of *Dacryodes excelsa* and *Sloanea* spp. (Carrington 1998), with numerous epiphytic ferns, bromeliads and lianas in the undercanopy. Secondary rain forests are characterized by *Miconia mirabilis*, *Cecropia schreberiana*,

and *Simaruba amara*. In disturbed areas that have been cleared primarily for logging and shifting agriculture, forest may be replaced by groves of palms or tree ferns. These degraded areas, especially steep slopes, are particularly vulnerable to accelerated erosion and recurrent landslides. Extensive cultivation of crops can exacerbate this problem (e.g. *Ananas comosus, Musa* spp., and *Citrus* spp.).

Within islands, microclimate can vary dramatically, according to the interacting factors of elevation, topography, and exposure. This in turn results in a corresponding multifarious pattern in vegetation cover. In addition, anthropogenic influences on land use considerably affect the composition of vegetation. However, based on exposure to key climatic elements, some generalisations may be made about the pattern of variation in vegetation type repeatedly observed across islands. Eastern coasts receive more rainfall than western coasts, which often fall under a rainshadow from the high relief of the mountain barrier ranges of the young Lesser Antilles. Accordingly, the altitudinal limits of eastern coastal vegetation zones are lower than on the western coasts. Eastern coasts also receive more salt-laden air from the prevailing north easterly trade winds, and thus eastern coastal littoral vegetation is characterised by salt-tolerant species, whilst western coasts are often drier and are dominated by dry scrub woodland (Beard 1948).

Dominica (15° 10' N, 61° 15' W) is one of the largest of the Lesser Antilles (754km^2), and is separated from the neighbouring islands of Guadeloupe and Martinique by deep sea channels. Its relief is characteristically variable, rugged and steep, with a range of high mountains forming the north-west to south-east backbone of the island. The island interior is covered by a largely intact tropical rain forest, historically unexploited due to inaccessibly steep terrain, which has prevented the extensive development of large-scale plantations. The highest point of the island, Morne Diablotin, reaches 1447m altitude and is often covered in cloud. The climate and vegetation of Dominica is heavily dependant on the varying amount of rainfall different areas receive. The high relief of the island, like others of the younger Lesser Antillean arc results in heavy rainfall (up to 9000 mm, among the highest accumulations in the world) in the higher, interior of the island (Lang 1967). The drier west coast receives around 1800 mm per year. A vegetation map for Dominica is presented in Fig. 1.4.

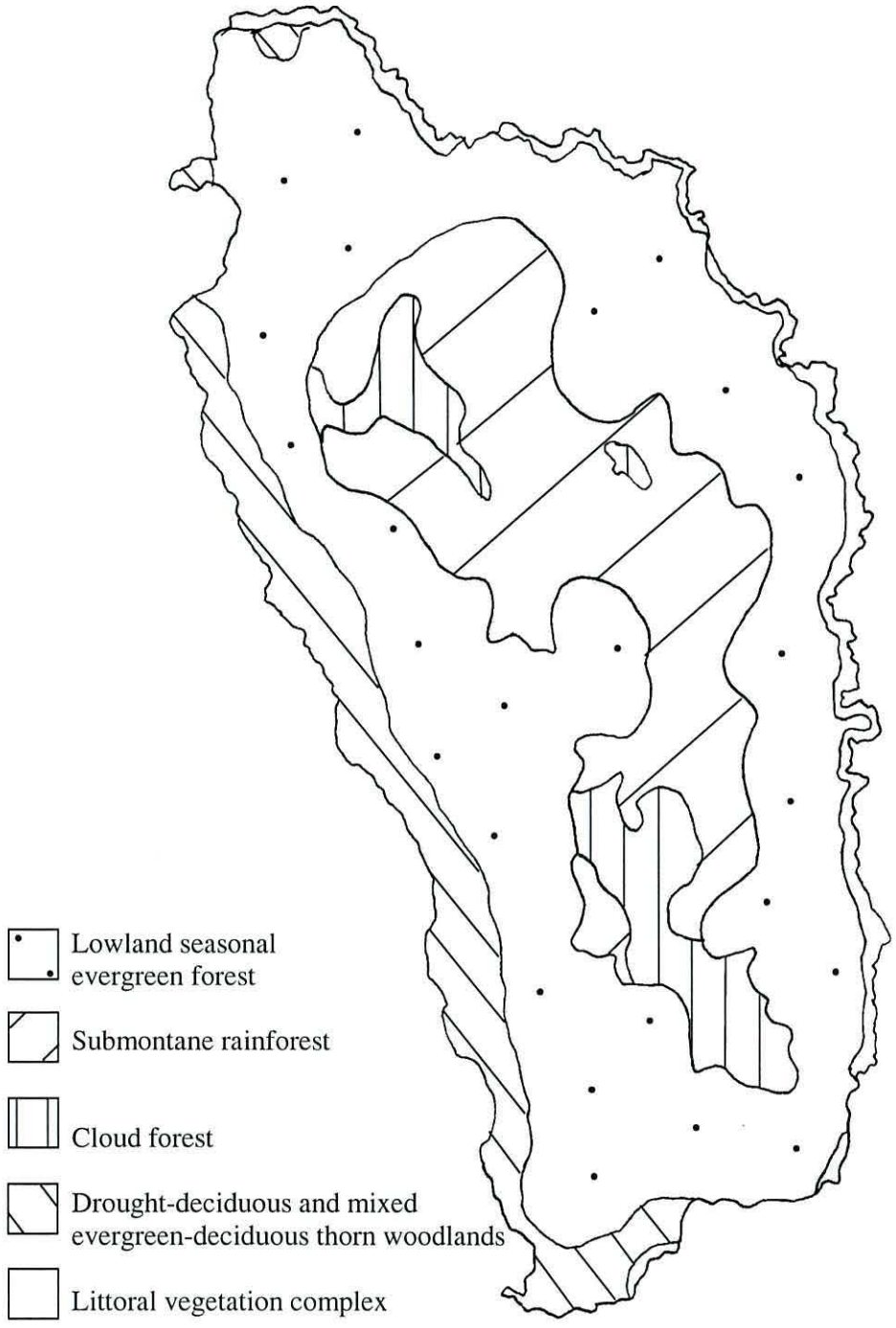

Figure 1.4.
Dominica vegetation zones. Redrawn from Evans (1986).

The natural, endemic anole on Dominica, *Anolis oculatus*, is the most commonly seen of all reptiles on the island, and is present in high densities in many of its lowland habitats. St Vincent (13° 15' N, 61° 12 W) with an area of 344 km^2 lies between the islands of St Lucia and Grenada. A rugged mountain range extends north to south from

La Soufrière, the island's active volcano, which erupted violently in 1812, and again in 1902. A mild eruption in 1971–72 created a volcanic dome in the crater lake, forming an island, which exploded during the most recent eruption in 1979. St Vincent receives 2000 mm per annum in coastal areas, 3800 mm per annum in the more mountainous interior, where tropical forest covers about 15% of the total land area. St Vincent has two endemic anoles, the small *Anolis trinitatus*, and the larger *Anolis griseus*.

Tropical seasons for the Greater Antilles follow a similar pattern to those in the Lesser Antilles, although the rainy season may begin earlier, in April and finish in November. As in the Lesser Antilles, the orographic effect of highland areas dominates the intra-island variation in precipitation, and is also affected by prevailing north easterly winds. The temperature ranges are typically slightly cooler than those quoted for the Lesser Antilles, ranging from an average of 23°C in January to an average of 28°C in August. Like the Lesser Antilles, soils are predominantly clay-based and vegetation is characterised by those species described for Lesser Antillean vegetation types (see above).

Puerto Rico (18° 15' N, 66° 30' W), the smallest and easternmost island of the Greater Antilles lies approximately 120 km east of Hispaniola, with an area of 9100m^2. The island extends approximately 180 km from east to west and 65 km from north to south. The mountainous interior of Puerto Rico is formed by a central mountain chain (the Cordillera Central) which rises to a maximum altitude of about 1338 m above sea level and transects the island from east to west. The karst region in the north west consists of limestone formations (sinkholes, caves, limestone cliffs, and other karst features) dissolved by water. Average annual precipitation in Puerto Rico ranges from less than 1000 mm on the southern coastal plain to greater than 5000 mm in the mountains and the north of the mountains, and averages about 2000 mm on the windward north coast of Puerto Rico. Most primary natural vegetation has disappeared, as virtually all of the available land on the island has undergone successive cultivation, excepting several protected mountainous forest reserve sites (e.g. Sierra de Luquillo in the north east). Seasonal deciduous forests are found in the southern hills, and dry woodland scrub and cactus savannas remain in coastal areas of the south. Puerto Rico is home to ten native *Anolis* species.

1.4.3 Anolis

Around 400 species are included in the *Anolis* genus (Reptilia: Sauria: Iguanidae), making it one of the largest and most diverse vertebrate genera, comprising 5-10% of present day lizard fauna (Williams 1969, 1983; Burnell & Hedges 1990). Around 140 of these species are found in the Caribbean, with an endemic on almost every island, the rest are dispersed across Central and northern Southern America (Powell *et al.* 1996). Their abundance and diversity, together with the intensity and variety of interspecific ecological relationships, are key features which have favoured the use of Caribbean anoles for empirical observations and as experimental models. Studies began on the genus in the Caribbean in the late 1950's (Etheridge 1959, Underwood, 1959) and *Anolis* have continued to be used in studies of niche theory (Gorman & Hillman 1977; Roughgarden *et al.* 1981; Pacala & Roughgarden 1982), biogeography (Williams 1969), and evolutionary behaviour (Stamps 1977; 1983). Inter-specific studies on Caribbean anoles have been used to investigate adaptive radiation and evolutionary ecology (Roughgarden & Pacala 1989; Losos 1990; Losos 1992; Losos *et al.* 1994; Losos 1995; Losos *et al.* 1997; Losos *et al.* 2000; Knox *et al.* 2001), whilst intra-specific studies have predominantly focused on geographical population differentiation (Lazell 1972; Schwartz & Henderson 1991; Malhotra & Thorpe 1997a, 1997b) and natural selection (Malhotra & Thorpe 1991a; 2000; Thorpe *et al.* 2005).

Anolis are relatively small-sized, generalist lizards (generally less than 80 mm snout to vent length, excepting giants), mainly insectivorous and are often found to occupy all microhabitats on an island (Roughgarden 1995). The males of many species have brightly coloured, extensible neck flap (dewlaps) that are used to communicate in territorial and sexual displays (Roughgarden 1995). In the Caribbean, anoles dominate the arboreal insectivorous niche, probably predation on anoles is relatively low on small islands (Roughgarden 1995). It has also been suggested that exothermic lizards may be more energetically efficient than warm-blooded birds, which could also account for the high densities of more than one individual anole per m^2 (Schoener & Schoener 1980). On larger islands and continents, predation reduces anole abundance and birds compete for occupation of the niche.

Molecular evidence (Hedges *et al.* 1992; Hass *et al.* 1993; Stenson *et al.* 2004) along with earlier comparative morphological analyses (Underwood 1959; Etheridge 1959) and biochemical investigations (Burnell & Hedges 1998; Gorman & Atkins 1969) show that the Caribbean herpetofauna are primarily derived from overwater dispersal events, rather than isolation following island fragmentation. Caribbean anoles derive from two colonising stocks from central and southern America, the first lineage colonised the Southern Lesser Antilles up to Martinique forming the *roquet* group, and the second *bimaculatus* lineage colonising the northern islands (Underwood 1959; Roughgarden 1995; Jackman *et al.* 1997; Stenson *et al.* 2004). Small (Lesser Antillean) island banks have just one or two species on them, whereas large (Greater Antillean) banks, have complex, competition-driven anole communities (Losos 1995). The study of these contrasting anoline faunas has been used as an example of colonisation and radiation when species are faced with competitive and environmental limitations (Haefner 1988; Losos 1995; Genet 2002).

1.4.4 Greater and Lesser Antillean anoles: adaptive radiation and intraspecific divergence

The Greater Antilles host 111 *Anolis* species, with up to 54 species on a single island (Powell *et al.* 1996). Although rates may vary dramatically between islands (Brown & Kodric-Brown 1977), evolution is generally much faster than immigration on small, oceanic islands (MacArthur & Wilson 1967). This, together with the relative slowness of lizard colonisation (Losos & Schluter 2000) and the considerable distances separating the island banks of the Greater Antilles, suggests that radiation, rather than repeated colonisation, is responsible for within-island anole diversity (Rand 1964). Phylogenetic work (reviewed in Jackman *et al.* 1999) has substantiated the key role of radiation processes and suggests that as few as 11 inter-island colonisation events are necessary to explain the distribution of Greater Antillean anoles (Jackman *et al.* 1997). The present day diversity in anoline fauna is therefore largely a result of within-island speciation events (Losos & Schluter 2000).

Adaptive radiation requires environmental heterogeneity together with moderately intense inter-specific competition to drive the completion of speciation (Losos 1995). With considerable heterogeneity on each island (due to their relatively large size) the

Greater Antilles provide an ideal arena for adaptive radiation. The role of competition as the driving force behind community evolution is supported by a large body of evidence for anole communities (reviewed in Losos 1994). On most of the Greater Antillean islands exist a set of six core habitat specialists (ecomorphs, Williams 1983), which are distinguishable in multiple morphometric traits that relate directly to functional capacity (Moremond 1979; Losos 1995).

Because of their historical dominance in their niche and relatively generalist diet (Knox *et al.* 2001), anoles have had the ability to specialise, resulting in many sympatric species (between which clear ecological differences have been described, Rand 1962), allopatrically separated species, and the evolution of many unique types of habitat specialists (Losos 1995). This has produced a repeated pattern of anoline species assemblages for each island that are morphologically adapted to different parts of the environment. The repetitive convergent evolution of these anole assemblages had been verified by phylogenetic studies, and provides robust evidence that variation in traits represents adaptation to habitation in different environments (Brown *et al.* 1991; Losos & Thorpe 2004). Puerto Rico presents an excellent example of the Greater Antillean island radiations with its ten climatically vicariant anoline species demonstrating morphological separation, reproductive isolation and ecological constraint (Losos & Thorpe 2004).

The Lesser Antilles, in contrast to the Greater Antilles (Roughgarden 1995), contain either one or a pair of endemic anoles on each island (Rand 1964). Molecular phylogenies have shown Lesser Antillean island pairs not to be recently divergent species (Stenson *et al.* 2004). Thus, there has been little or no internal speciation on the Lesser Antilles, and each anole is likely to have arrived separately by independent cross-water colonisations (Schneider *et al.* 2001). The most plausible explanation for the absence of speciation on these islands is their small size (Losos 1996). On the Lesser Antillean islands containing two species, anoles are characterised by body size divergence rather than distinct habitat adaptation (Schoener 1970; Roughgarden 1992; Roughgarden 1995). A difference in body size (by the factor of at least 1.3) enables partitioning of prey by size, thereby reducing competitive effects (Haefner 1988).

Although adaptive radiation has not occurred within one species islands, there are some instances of high interpopulational morphological variation that represent adaptation to environmental gradients and different habitats, notably in Guadeloupe, Martinique and Dominica (Malhotra & Thorpe 1991b; Giannasi *et al.* 2000). This extensive intraspecific divergence is at least as much as the interspecific divergence within Greater Antillean anoline species (Knox *et al.* 2001). Such high variation in sub-specific populations on discreet islands has provided an opportunity to investigate natural selection (Malhotra & Thorpe 1997b). The Lesser Antilles are highly heterogenous on geological and environmental levels within a relatively small area, a potential selective force for adaptive radiation. The occurrence of parallel trends in colouration on other Lesser Antillean anole species provides further evidence for the importance of environmental selective pressures for intraspecific adaptation (Thorpe & Stenson 2003).

1.4.5 Anolis *introductions*

Anoles have been widely transported throughout the Caribbean island chain both by their own dispersal mechanisms (mainly via hurricanes, Calsbeek & Smith, 2003) and more recently unintentionally through anthropogenic transportation (Williams 1977). The most striking examples of anole human mediated dispersal include the introduction of *A. carolinensis* in the Bonin islands, Japan in the 1960's (Hasegawa *et al.* 1988) and of *A. sagrei* recorded in Taiwan in 2000 (Norval *et al.* 2002). Establishment may be more likely for recent introductions because human-mediated introductions often contain more individuals than natural propagules (Goel & Richter-Dyn 1974), and anthropogenic habitat alterations may enable easier spread and penetration of novel environments (Williams 1969).

Table 1.1. (following page)

Success of Caribbean *Anolis* introductions (altered from Losos *et al.* 1993)

a. Number in parentheses is the total number of *Anolis* species in the local community at the time of invasion. In some cases of multiple introductions, uncertainty exists concerning this number, so a range of numbers is given.

b. For Greater Antillean anoles defined as different ecomorph types (Williams 1983; Losos 1990), members of the same ecomorph were considered ecologically dissimilar if they differed in thermal microclimate. Lesser Antillean anoles that differed in body size by a ratio of 1.65 to be considered dissimilar.

Island	Introduced species	Resident species a	Ecologically similar b	Result	References
Anguillita	pogus	gingivinus (1)	yes	Failed	Roughgarden *et al.* 1984
Bermuda	Ieachi	grahami (1)	no	Widespread	Wingate 1965
Bermuda	extremus	grahami (1)	yes	Marginal	Wingate 1965
Cayman Brac	maynardi	sagrei (1)	no	Marginal	Losos, unpublished data
Florida	distichus	carolinensis (1 2)	no	Widespread	Wilson and Porras 1983
Florida	sagrei	carolinensis (1-2)	no	Widespread	Wilson and Porras 1983
Florida	equestris	carolinensis (2-3)	no	Widespread	Wilson and Porras 1983
Florida	conspersus	carolinensis (1)	yes	Failed	King and Krakauer 1966
Florida	cybotes c	sagrei (2-3)	yes	Marginal	Wilson and Porras 1983
Florida	cristatellus	sagrei (2-3)	yes	Marginal	Wilson and Porras 1983
Florida	garmani	equestris (3-4)	yes	Marginal	Wilson and Porras 1983
Grand Bahama	carolinensis	sagrei (1-2)	no	Marginal	Losos, unpublished data
Grand Bahama	distichus	sagrei (1 2)	no	Widespread	Losos, unpublished data
Grand Cayman	sagrei	conspersus (1)	no	Widespread	Losos *et al.* 1993
Great Abaco	distichus	sagrei (1)	no	Marginal	Schoener, unpublished data
Hispaniola	cristateIlus	cybotes (4)	yes	Marginal	Fitch *et al* 1989
Hispaniola	porcatus	chlorocyanus (4)	yes	Marginal	Powell *et al.* 1990
Jamaica	sagrei	lineatopus (5)	no	Widespread	Williams 1969
Louisiana	sagrei	carolinensis (1)	no	Marginal	Thomas *et al.* 1990
St. Lucia	wattsi	luciae (1-2)	yes	Marginal	Corke 1987
St. Lucia	extremus	luciae (1-2)	yes	Marginal	Gorman 1976
St. Maarten	bimaculatus	gingivinus (2)	yes	Marginal	Powell *et al.* 1992
Trinidad	aeneus	trinitatis (2)	yes	Marginal	Gorman *et al.* 1971; Gorman & Boos 1972
Dominica	cristatellus	oculatus (1)	yes	Widespread	Eales *et al.* 2008b
St. Vincent	sagrei	trinitatus (2)	yes	Marginal/Failing	Eales *et al.* 2008c

Table 1.1.

Success of Caribbean *Anolis* introductions (altered from Losos *et al.* 1993)

The nature of *Anolis* community formation in the Caribbean exposes the vulnerability of Lesser Antillean islands to Greater Antillean anole invasion. Whilst solitary Lesser Antillean anoles have not been exposed to congeneric competition, and two-species Lesser Antillean islands are generally influenced by low levels of competition, Greater Antillean species have evolved under strong inter-specific effects to become competitively robust (Losos 1992). In a review of anole introductions in the Caribbean by Losos *et al.* (1993), seven from a total of 23 documented cases became widespread across the island. An amended, updated table from Losos *et al.* 1993 is presented in Table 1.1. In each of the seven widespread *Anolis* introductions, the introduced and native species were of different ecomorph types, and wherever introduced anoles failed to establish, the species was ecologically similar to an existing native anole (Losos *et al.* 1993). Thus, the presence or absence of an ecologically similar native species may be an important determinant of colonization success or failure (Table 1.1). This, together with variations in inter- and intra- island habitat type, in the amount of human environmental disturbances and in the number of individuals transported, suggests that each *Anolis* introduction must be considered on an individual basis to successfully assess the potential for establishment and ecological impact. Greater Antillean anoles introduced to Lesser Antillean islands have only recently been documented (Greene *et al.* 2002; Malhotra *et al.* 2007), although invasions among Greater Antillean and Lesser Antillean islands have occurred separately (Fitch *et al.* 1989; Losos *et al.* 1993; Giannasi *et al.* 1997).

A. cristatellus, the Puerto Rican Crested anole, is the most common *Anolis* found in mesic-xeric habitat on its native Puerto Rico. It is a medium sized, trunk-ground ecomorph (Williams 1983) with pronounced sexual dimorphism (average male snout to vent length is 1.45 times that of females, Fitch *et al.* 1989). Female *Anolis* reach sexual maturity between two and 12 months (Campbell 1973; Lee *et al.* 1989; Fitch *et al.* 1989), which, when coupled with a long breeding season for *A. cristatellus* shows a capacity for rapid population increase (Fitch *et al.* 1989). The lizard has been observed feeding opportunistically on insects attracted to nocturnally lighted urban areas (Garber 1978), and examination of stomach contents together with field observations have revealed evidence of intra-guild predation and cannibalism (Fitch *et al.* 1989; Gerber 1999). The species presents a wide range of aggressive behavioural displays including extension of the throat fan (dewlap) and lateral face off. The opportunistic, generalist

and aggressive characteristics of *A. cristatellus* have led to its classification into a "colonising phenotype" as termed by Hertz (1983). The species has rapidly and successfully established populations in the Dominican Republic (Fitch *et al.* 1989) and southern Florida (Brach 1977).

Following introduction to the Dominican Republic in the 1920's (Grant 1956) at the port in the southeast of the island, by 1989 *A. cristatellus* had spread to cover approximately 12km^2 of human influenced land, although not penetrating any of the surrounding dense vegetation (Fitch *et al.* 1989). Both native species (*A. distichus* and *A. cybotes*) show low abundance in areas where the introduced species is present (Fitch *et al.* 1989), suggesting that *A. cristatellus* dominates the spatial partitioning of its sympatric *Anolis* habitat (Fitch *et al.* 1989). Across the introduced *A. cristatellus* range in Florida, the native *A. carolinensis* is uncommon or absent from areas in which *A. cristatellus* is abundant (Saltzburg 1984).

A. cristatellus was introduced to Dominica around 1998-2000 (Plate I; IIa). In 2002 a preliminary survey to assess the extent of the invasion was carried out at 60 locations across the island. At this time, *A. cristatellus* was present approximately four km north of Deepwater Harbour dock along the coast. Maximum extent of range was estimated by extensive surveying for *A. cristatellus* along coastal and inland roads. This information was recorded at intervals since the discovery of the introduction, and is presented in Fig 1.5. The introduced anole was found at high densities around the Deepwater Harbour dock, suggesting an anthropogenic mode of transport via shipping containers. Following regular annual surveying, the rate of spread of the introduced species is estimated at a minimum of one km per annum from the estimated time of introduction until 2002. A detailed survey in 2003 including an additional 23 localities found that *A. cristatellus* was present approximately one km inland from Massacre to an elevation of 150m. The south coast limit was first recorded in 2005, approximately eight km south of Deepwater Harbour, Fig. 1.5. Morphological work in 2003 on the introduced species revealed significant phenotypic differences between individuals collected from low and high altitude (Kay 2003), an adaptive pattern that is similar to that shown by the native anole *A. oculatus* (Malhotra 1992). Positions of the estimated north coast, east/high altitude and south coast range limits are given in Appendix I.

In the same 2003 study, only large *A. oculatus* males were observed in sympatry with the introduced anole, which may be interpreted as a response to intense competition or intraguild predation, consistent with evidence of intra-guild predation by *A. cristatellus* (reviewed by Gerber 1999). This evidence suggests native anole displacement may predominate on Dominica, as has been the case in other *A. cristatellus* introductions. However, because *A. oculatus* is known to occupy a wide range of habitats, being geographically variable in both morphology and ecology (Malhotra & Thorpe 1997a), it is also possible ecological separation may prevail, enabling areas of sympatry.

Of all the Caribbean anoles, the brown anole (*A. sagrei*) is perhaps the most widespread and successful at colonisation (Williams 1969). A native of the Bahamas, Cuba and the Cayman islands (Campbell 1996), introduced populations have been reported in Jamaica (Williams 1969), Belize (Rodriguez Schettino 1999), Grenada (Greene *et al.* 2002), Hawaii (McKeown 1996), Mexico (Lee 1992), the states of Florida (Bell 1953), Texas (Conant & Collins 1991), Louisiana (Steven & Lance 1994), Georgia in the US (Campbell 1996; Reznick & Ghalambor 2001), and most recently, Taiwan (Norval *et al.* 2002). Invasive populations of *A. sagrei* can reach high population densities, expand their range rapidly (Losos *et al.* 1993) and have been shown to be competitively superior to native congenerics (Campbell 1996).

The widespread abundance of the introduced *A. sagrei* in Florida (Bell 1953) has displaced the native *A. carolinensis* from some peripheral native habitats. Here, *A. sagrei* has shown high levels of geographical morphological heterogeneity (Lee 1985), a phenomenon which has also been shown for the Bahamas, where the changes are likely to be a response to local ecological conditions (Losos *et al.* 2000). Within ten years of introduction on Grand Cayman, *A. sagrei* has expanded its range over most of the island, and is now more common in some habitats than the native anole, *A. conspersus* (Losos *et al.* 1993). The success of the colonisation may have been facilitated by interspecific differences in body size, perch height, and microclimatic preference (Losos *et al.* 1993).

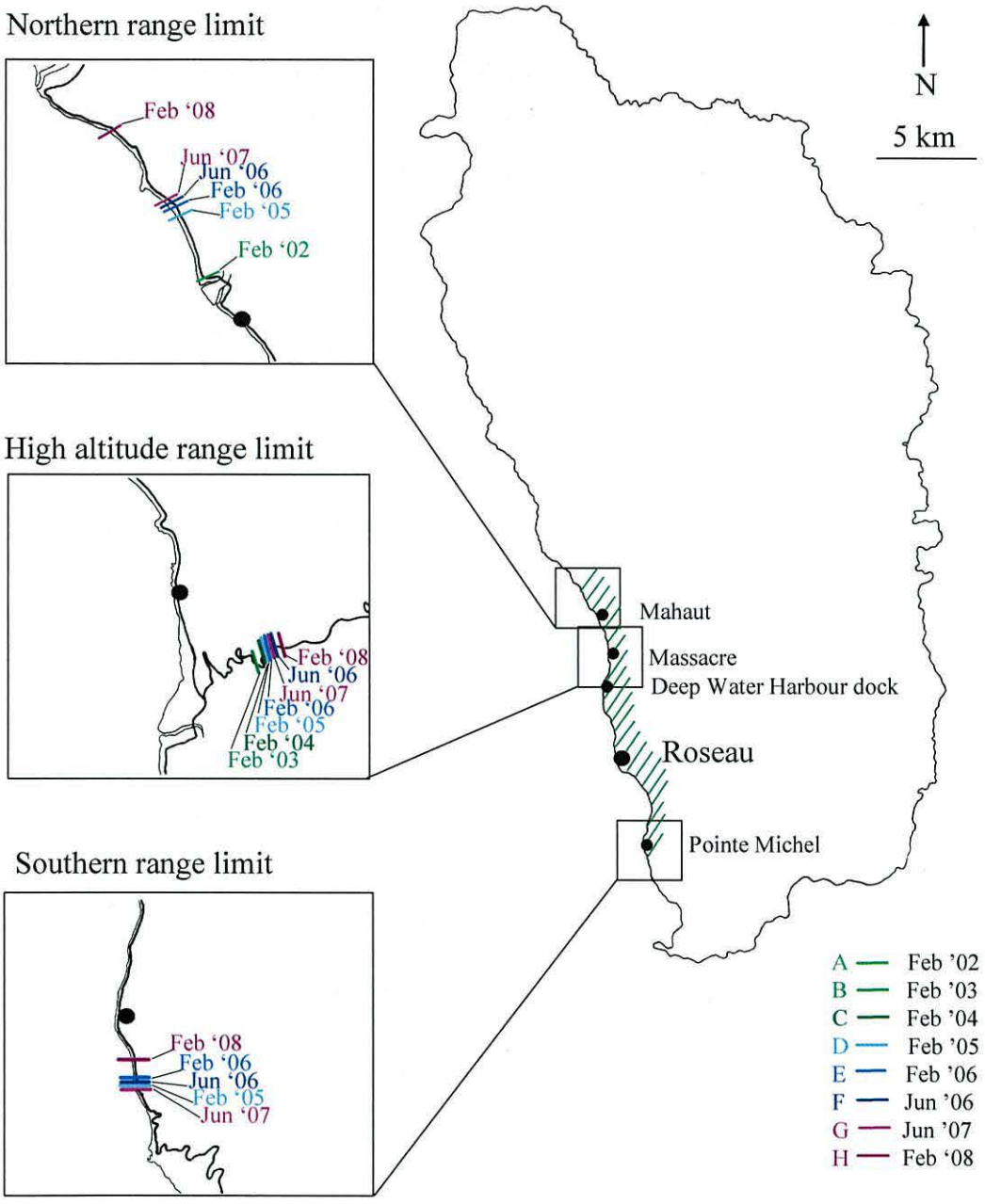

Figure 1.5.

Map of Dominica showing estimated maximum range extent of *A. cristatellus* since introduction *c.* 1998-2000 (green hatched area). Insets (1:24000) show range limits recorded between 2002- Feb 2008 for northern, southern and high altitude range limits.

In 2003, *A. sagrei* was reported at the main import dock at Campden Park, St. Vincent, 3km north east of the capital of Kingstown, Fig. 1.6; Plate IIb. The dock routinely receives imports from throughout the Lesser Antilles, Central America and Florida. In 2005, *A. sagrei* was present in a small, anthropogenically disturbed area (approximately 400m^2) adjacent to the dock, Fig. 1.6. The discovery of *A. sagrei* in St. Vincent is perhaps the most recent example of human mediated *Anolis* introductions in the Lesser Antilles.

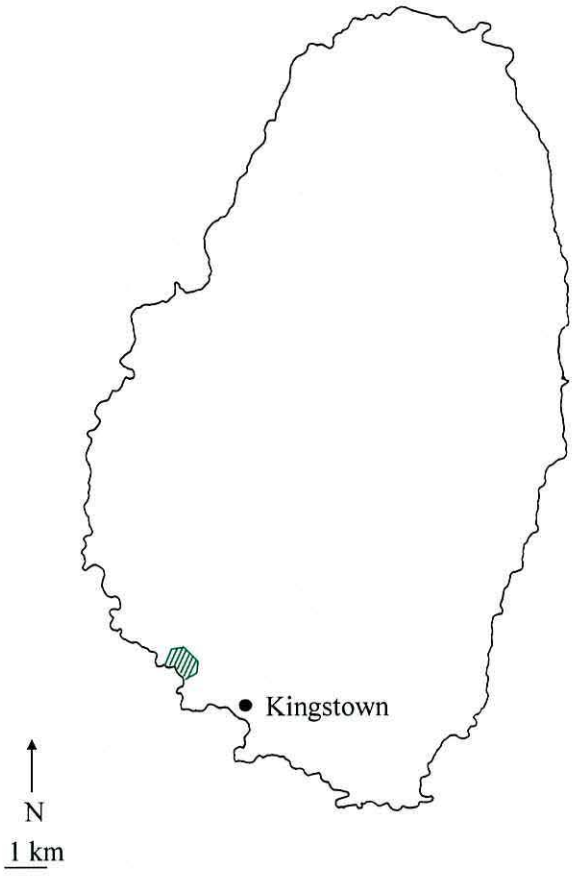

Figure 1.6.
Map of St. Vincent showing February 2005 range (within green hatching) of introduced *A. sagrei.* Range observed in June 2007 not shown, due to extremely low population density.

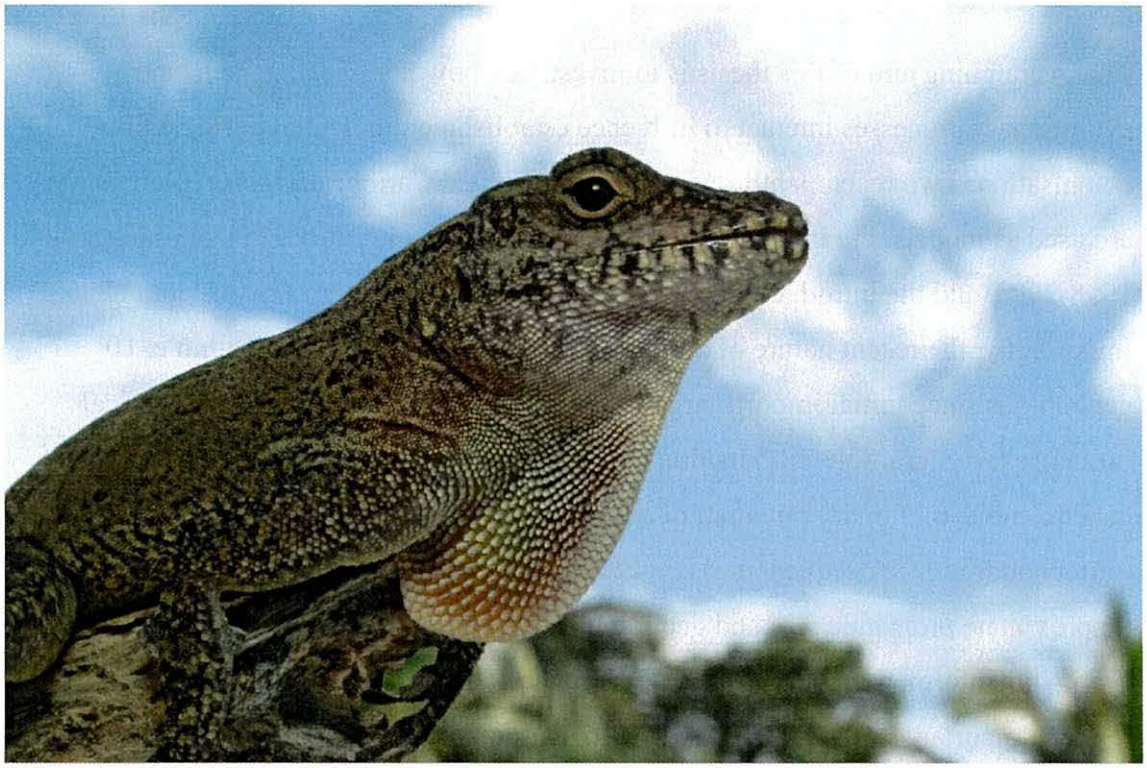

Plate II a).

Anolis cristatellus from Botanical Gardens, Dominica. Photograph by Roger Thorpe.

Plate II b).

Anolis sagrei from Grenada. Photograph by Yann Surget-Groba.

1.5 General Aims

The overarching aim of this thesis is to investigate how historical, genetic and evolutionary processes interact to influence establishment potential during early colonisation. Two recent, anthropogenically-mediated Caribbean *Anolis* colonisations are used as models: the Puerto Rican *Anolis cristatellus* introduced to Dominica around 1998-2000, and the introduction of the Cuban native *A. sagrei* to St. Vincent *c.* 2003. Importantly, the recent nature of these *Anolis* colonisations (studied within *c.* 10 generations since initial import) largely enables the exclusion of various historical influences (e.g. multiple introduction events and genetic drift) which can affect the genetic and evolutionary potential of colonisations. A molecular approach (mitochondrial DNA sequencing) is used to reconstruct the introduction history (approximate propagule size and geographical origin) of the colonising populations. Using this information, the thesis aims to investigate how colonisation history (and the relative importance of stochastic and ecological forces) may shape the genetic characteristics and evolutionary potential of introduced populations during early colonisation.

This thesis aims to detect a signal for a genetic founder effect within introduced populations during early colonisation, using microsatellite data. Analyses of this population genetic data from source, native and introduced populations are used to estimate the potential source of the genetic diversity in introduced populations. Following previous studies that have demonstrated rapid responses to environmental selective pressure in island *Anolis*, both genetically-based (Malhotra & Thorpe 1991a) and plastic (Losos *et al.* 1997), the recent model colonisations offer a rare opportunity to investigate the potential rapid adaptive responses to novel environmental selection pressures within an introduced range. Using common garden experiments to test the genetic basis for geographic variation in phenotypic characters, inferences are made on how genetically-based and plastic responses to selective forces can jointly influence establishment potential.

CHAPTER 2

Rapid adaptation of an invasive population: *Anolis cristatellus* in Dominica

2.1 Abstract

Rapid adaptation to differing environmental selective pressure within the range of an invasive species has rarely been documented. This study shows variation in adaptive phenotypic traits among invasive populations of *Anolis cristatellus* at elevation extremes of the introduced range in Dominica, Lesser Antilles within ten generations. This trend has been linked to ecological selective pressures that vary with elevation, and has also been documented in the native congener. Common garden experiments show that the putatively adaptive trait variation is retained in F1 progeny, and that estimated heritabilities are high for the measured traits. Colonisation history (multiple introductions, differential dispersal of pre-adapted haplotypes) can be excluded as a basis for the elevation patterns on Dominica by mitochondrial DNA analysis. The results provide evidence that genetically based adaptive traits in *A. cristatellus* are likely to be responding to ecological selective pressure within around ten generations of exposure to such pressures. The genetic capacity for adaptive responses may derive from a weak founder effect, demonstrated by a moderate founder population size and presence of considerable genetic diversity within Dominica. In addition, the genetic diversity within demographically bottlenecked populations of *A. cristatellus* may be augmented by life history characteristics (sperm storage and multiple paternity) of *Anolis* lizards.

2.2 Introduction

An increased awareness that introduced species are repeatedly exposed to novel selective pressures has led to a rise in the number of studies that provide evidence for adaptation to ecological conditions in introduced populations (Costa-Pierce 2003; Parker *et al.* 2003; Maron *et al.* 2004; Rasner *et al.* 2004; Yeh 2004; Ayllon *et al.* 2006). A significant ecological driver of rapid adaptive divergence is a change in the biophysical environment, a recurrent feature in the establishment of populations in previously unoccupied habitat (Reznick & Ghalambor 2001). Moreover, environmental conditions may vary within the introduced range, presenting further opportunity for localised adaptive responses. Plastic responses to biotic and abiotic conditions are also likely to play an important role in providing appropriate and rapid phenotypic responses to ecological selection pressures. Investigations into the ability of introduced species to show adaptive responses to environmental heterogeneity within the new range provide an invaluable opportunity to predict the spread of localised adaptation, and thus, their invasion potential. Consequently, adaptation studies are likely to be important in directing management strategies and conservation focus in species introductions (Costa-Pierce 2003; Mack *et al.* 2000). These investigations are also relevant to wider conservation issues primarily concerning demographically bottlenecked populations that are closed to migration and which are under selective pressure to adapt to alterations in environmental conditions; for example, populations of endangered species isolated within human-altered or disturbed habitats (Frankham *et al.* 2002; Jehle &Arntzen 2002; Smith & Wayne 1996).

The ability of populations to adaptively respond to selective pressure depends critically upon the availability of genetic diversity (Fisher 1930; Reznick *et al.* 1997). However, many introduced populations are likely to have undergone a demographic bottleneck, which has been associated with loss of genetic diversity, the 'founder effect' (Mayr 1963). A fuller understanding of how aspects of early-stage colonisation history may affect the genetic capacity for adaptive responses is clearly required. These aspects include founder effects and founding population size (which can influence genetic diversity available for adaptive responses), the degree of genetic admixture from

multiple introductions and colonisation patterns by locally pre-adapted genotypes. Such investigations require both thorough genetic characterisation of native and introduced ranges, and a comprehensive knowledge of introduction history. However, many empirical studies are unable to resolve the influence of colonisation patterns on the introduction of genetic diversity because introduction histories for the majority of species are poorly understood.

While genetically-based phenotypic shifts between source and introduced populations may potentially be based on genetic drift arising from founder effects (Mayr 1954; Templeton 1980; Carson & Templeton 1984, Caño *et al.* 2008), phenotypic trait divergence within introduced ranges is a more direct demonstration of adaptive potential. Evidence for adaptation is particularly strong when correlated with environmental conditions known to affect those traits, or when introduced populations show clines that converge with those present in native populations. Latitudinal clines in size and fecundity of introduced St. John's Wort *Hypericum perforatum* follow those documented in native populations, and are associated with the broad-scale abiotic conditions experienced across the introduced North America range (Maron *et al.* 2004). However, for the majority of species introductions, the occurrence of multiple introduction events cannot be discounted, and genetically-based adaptive trait divergence may be a result of colonisation patterns or differential dispersal from multiple introductions of different pre-adapted genotypes (e.g. Brassicaceae *spp* [Hurka *et al.* 2003], brown alga *Undaria pinnatifida* [Voisin *et al.* 2005]) rather than via natural selection. Hence, empirical examples of adaptive responses to within-introduced range environmental heterogeneity have been rare (although see Johnston and Selander [1971]; Carroll &Boyd [1992]; Maron *et al.* [2004]; Phillips *et al.* [2006]).

The recent introduction (between 1998-2000) and subsequent establishment of *Anolis cristatellus* on Dominica, Lesser Antilles, provides an opportunity to investigate evolutionary divergence in adaptive phenotypic traits within an introduced range in an early-stage species introduction. Since introduction at the port of Deepwater Harbour in the south-west of the island, *A. cristatellus* has extended its altitudinal range from sea level to approximately 180m above sea level (Fig. 2.1).

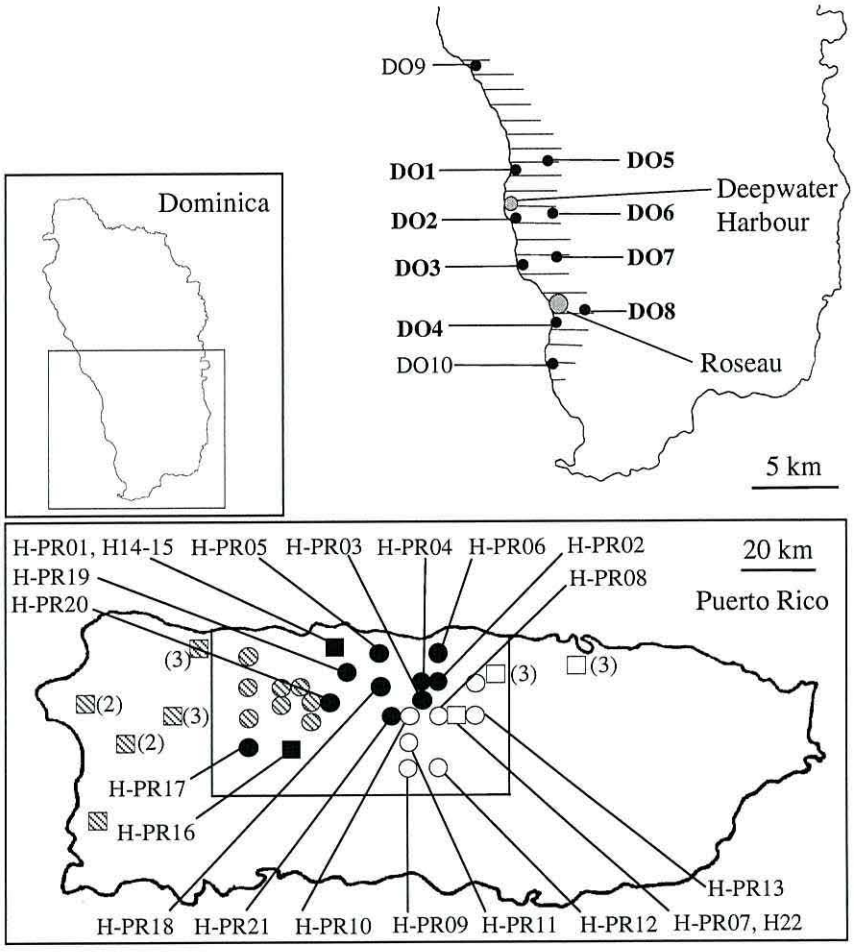

Figure. 2.1

Map of Puerto Rico and Dominica showing sample location with Dominica inset showing area of expanded detail. Sampling sites for the phylogenetic study in Dominica are DO1-10, while sites also sampled for the common garden experiment are labelled in bold type (low elevation sites are DO1-4; high elevation sites are DO5-8). Site positions and elevations are given in Appendix I. Horizontal hatching indicates the geographical range of *A. cristatellus* in Dominica as recorded in June 2007. Puerto Rico map shows sampling localities for unique NADH2 haplotypes identified in Fig. 2.3. Circles denote localities sampled by J. Eales, squares denote localities sampled by R. Glor (unpublished data). Shading/colouring of symbols indicates clade as defined by the phylogram in Fig. 2.3 (black= Central West clade; white= Central East clade; diagonal hatched= West clade). Labels H-PR01 to H-PR22 indicate sampling areas for the Puerto Rico haplotypes close to Dominican clades in the Bayesian phylogram. Numbers adjacent to symbols denote the number of haplotypes represented per locality. The

boxed area indicates the extent of the geographical area sampled for this study (79 localities, two individuals sampled from each locality).

Anolis are known to be highly phenotypically variable (Thorpe &Malhotra 1996; Malhotra & Thorpe 1997a; Thorpe 2002), and empirical data from endemic *Anolis* across the Lesser Antilles show that body size, colour pattern and scalation traits vary clinally with elevation (Malhotra &Thorpe 1991b; Malhotra 1992; Harris 1995; Malhotra &Thorpe 1997a). Habitat transition (e.g. from xeric woodland to transitional rainforest in the south-west of Dominica) along these altitudinal clines suggest that the variation in scalation traits may represent phenotypic adaptation to ecological and microclimatic conditions (Malhotra &Thorpe 2000; Thorpe &Stenson 2003; Losos & Thorpe 2004). The number, size and shape of lizard scales have been shown to vary with a number of these conditions (including temperature and rainfall, [Lister 1976; Thorpe & Baez, 1987; Malhotra & Thorpe 1997b; Calsbeek *et al.* 2006]), and are vitally important for the maintenance of water balance in thermo-conforming reptiles (Horton 1972; Soulé & Kerfoot 1972; Daltry *et al.* 1998; Alibardi 2003). Thus, scalation traits in *Anolis* are expected to be under substantial selective pressure along altitudinal gradients. Molecular investigations have excluded historical processes as an explanation for the variation in measured *Anolis* phenotypic characters along these gradients (Malhotra & Thorpe 1997b; Ogden & Thorpe 2002; Stenson *et al.* 2004). Heritability of scale number in *Anolis sagrei* has been demonstrated in a recent natural selection study (Calsbeek *et al.* 2006), and common-garden experiments in the native Dominican anole, *Anolis oculatus*, demonstrate that scalation traits are under significant genetic control (Thorpe *et al.* 2005). Thus, scale number is an adaptive trait that is likely to respond to ecologically-based selection.

This study uses common-garden experiments, which facilitate the distinction of genetic and plastic processes, to investigate and explore the genetic component to phenotypic divergence in scale number at the altitudinal boundaries of the introduced range of *A. cristatellus* on Dominica. Mitochondrial DNA phylogenetic information is used to determine the origin of Dominican *A. cristatellus*, and is combined with previously published microsatellite data (Eales *et al.* 2008a) and effective founder population size estimates to infer the genetic capacity for adaptive responses to selective pressure by the introduced population (Sakai *et al.* 2001). MtDNA evidence is also used to assess the geographical distribution of genotypes in Dominica. The specific questions we address

are: first, does phenotypic trait divergence exist between altitudinal extremes of the introduced range, and if so, is there evidence that this trait divergence is heritable? Second, do genotypes in Dominica originate from altitudinally variable sites in the source range (Puerto Rico), and if so, can the phenotypic divergence in Dominica be attributed to geographical segregation of these locally pre-adapted genotypes? Third, are estimates of founder population genetic diversity consistent with genetically-based trait variation in the introduced population?

2.3 Methods

2.3.1 Common garden experiment and analysis

Anolis cristatellus in Dominica has reached an approximate maximum elevation of 180m, whilst the endemic anole, *A. oculatus,* occurs at elevations up to 600m (Malhotra 1992), or even higher. Gravid female *A.cristatellus* were sampled from eight localities, DO1-8 (paired across four altitudinal transects) to represent the current altitudinal extremes of the introduced range (Fig. 2.1). Four low elevation sites ranging between 3 and 38m (mean 25.25m) above sea level and four high elevation sites ranging between 128 and 176m (mean 151m) above sea level were included in the sampling regime. As these four transects are distributed down the south-western sector of the island, latitudinal variation could also be controlled for.

Females from all localities were kept in identical constant conditions in captivity. Eggs and the resulting offspring were incubated and reared at identical, constant light, temperature and humidity conditions over a period of four months. Females and their resulting offspring were measured for two scalation characters: the number of scales between the midline of the ventral surface to the midline of the dorsal surface (circum-trunk scales), and the number of ventral scales between the limb axillae (ventral scales). Scale counts were undertaken with locality information shielded, repeated twice and an average of the two counts used for subsequent analysis. Where more than one offspring was produced per female, the means of scalation characters were calculated for the siblings. A lack of a significant difference in scalation characters between (maternal) parent and F1 progeny was tested using a three-factor ANOVA with elevation,

Plate III a).

Circum-trunk scale number measurement

Plate III b).

Ventral scale number measurement. *Photographs by J. Eales*

transect and generation as fixed factors. Second, a significant morphological differences between low and high elevation sites for F1 progeny was tested using a two-factor ANOVA, with elevation and transect as fixed factors. Transect was included as a fixed factor to control for the potential effect of latitude. Heritabilities for each of the scalation traits were calculated using parent-offspring regressions of scalation characters as implemented by Åkesson *et al.* (2007). Since only the maternal trait value was measured, the single parent–offspring regressions estimate half the heritability (h^2) (Falconer 1989; Lynch and Walsh 1998). Although the limitations of this approach which include potentially inflated heritability estimates due to maternal effects are recognised, the regression slope is still a useful estimator of heritability for the purpose of this study.

2.3.2 Molecular sample collection

To resolve the native-range geographical origin of *A. cristatellus* introduced to Dominica, 98 non-invasive tail tip biopsies were collected from ten sites across the introduced Dominican range (DO1-10, Fig. 2.1) and preserved in 95% ethanol. Information from a mtDNA phylogeny of Puerto Rican *A. cristatellus* placed a haplotype from the introduced Dominican range within a cluster of haplotypes from central Puerto Rico (R. Glor, unpublished data). Using this information, sampling in Puerto Rico was restricted to seventy-nine localities in central Puerto Rico, and two individuals were sampled from each locality (Fig. 2.1).

2.3.3 DNA extraction and sequencing

Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol for animal tissues. Approximately 945bp of mtDNA from the NADH2 gene was amplified using the primers H5730 (Glor *et al.* 2004) and L4437b (Macey *et al.* 1997). PCR reactions were carried out in 20µl volumes, containing 20ng template DNA, 1.5mM of $MgCl_2$, 2µl of Thermophilic DNA Polymerase 10x buffer, 0.2mM each dNTP, 0.5µM of each primer and 0.5U *Taq* Polymerase (New England BioLabs). PCR amplifications were performed in an MJ Research PTC-200 thermal cycler (Waltham, MA) using the following temperature cycling profile: a 5 min

denaturation step at 94°C followed by 40 cycles of denaturation for 30s at 94°C, annealing for 30s at 55°C and extension for 60s at 72°C. A final step at 72°C for 5 min followed the 40 cycles. PCR product was purified from agarose gel using the QIAquick gel extraction kit (Qiagen). Unincorportated nucleotides and primers were removed using 10xSAP (Promega) and Exonuclease I (New England BioLabs), following manufacturer's protocol (Promega). The forward and reverse primers were used for sequencing along with a specifically designed sequencing primer (L4280 5´-TTAACACAAGCAGCRGCMTCTGC-3´), using dye-labelled terminators (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit), and subsequently run on an ABI Prism 377 DNA sequencer.

2.3.4 Phylogenetic analysis

Mitochondrial sequence data collected for this study was combined with 65 mtDNA haplotypes for Puerto Rico, and five *Anolis* genus outgroups provided by R. Glor (unpublished data), after excluding replicated haplotypes to reduce computational time. Sequences were aligned using CODONCODE ALIGNER 1.2 (CodonCode Co.), and translated into amino acid sequences to check for the presence of stop codons which might indicate the amplification of pseudogenes. McDonald & Kreitman's (1991) test was used to evaluate the possibility of non-neutral evolution of the NADH2 gene (Ballard & Kreitman 1995). The genetic data analysis software package MRBAYES version 3.1 (Huelsenbeck & Ronquist 2001) was used to reconstruct a phylogenetic tree by Bayesian methods. All sites were equally weighted. The model of evolution used for Bayesian analysis was estimated by MODELTEST 3.0 (Posada & Crandall 1998) and the parameter values were estimated by MRBAYES. Four chains were run, with 2 million generations, sampling the chains every hundred generations. Time to stationarity was estimated by plotting tree log-likelihood score against generation number and determining the number of generations until the values reached an asymptote. A majority rule consensus tree ('Bayesian' tree) was calculated from the posterior distribution of trees, and the posterior probabilities calculated as the percentage of samples recovering any particular clade (Huelsenbeck & Ronquist 2001). Three further independent Bayesian analyses were run to check for local optima. Since multiple haplotypes from Puerto Rico were present in Dominica, the hypothesis that pre-adapted genotypes were responsible for any observed altitudinal phenotypic clines in Dominica

needed to be excluded. A chi-square contingency table was used to evaluate whether the distribution of haplotypes was significantly different among low and high elevation localities in both Puerto Rico and Dominica (Sokal & Rohlf 1995).

2.3.5 Founder population size

A minimum estimate of effective founder population size can be obtained both from mtDNA and microsatellite allele frequency data (Eales *et al.* 2008a). Since the Dominica population is very recent and assumed to have undergone negligible mutation, methods that incorporate models of evolution to estimate effective population size are inappropriate and of limited value. Furthermore, although the number of generations may be inferred relatively reliably for Dominican *A. cristatellus*, an estimate of current population size, which is necessary for many effective population size simulations, may be grossly inaccurate. The number of alleles (n) within the most variable locus in the Dominican population provides a minimum estimate of the effective founder population size (Rasner *et al.* 2004). Since there is no evidence to suggest that secondary introductions of *A. cristatellus* in Dominica have taken place, and *de novo* mutations are likely to have had a trivial impact, $n/2$ is the minimum estimate for the effective founding population size. The true number of founders is likely to be significantly greater than this minimum estimate because it is unlikely that each individual founder was heterozygotic at all loci. Furthermore, the allele frequency distribution of source populations is likely to alter the probability of founder populations containing n alleles. To obtain a more reliable estimate of minimum effective founder population size, a simulation program (written by R. Glor) was used. A specified number of individuals (between 1-100) were sampled from the allele frequency distribution of source populations. This was repeated 10,000 times, and the proportion of times that at least n alleles were contained within the sample was calculated. Although the simulation provides a minimum effective founder population size estimate, the true number of founders is likely to be higher since it is probable that the founder population comprised multiple individuals carrying the identified alleles. A lower bound estimate of founding population size can also be obtained from mtDNA data, since the number of haplotypes present in introduced populations is equal to the minimum number of sexually mature females present in the founding population. This figure is also likely to be an underestimate, since it is likely that multiple individuals

with the same mtDNA haplotypes were present in the founder population. The estimate of effective founder population size using mtDNA haplotype information is also only an estimate of the number of females in the founding population.

2.4 Results

2.4.1 Common garden analysis

Offspring were reared from between seven and 16 females sampled from each of the eight localities. A histogram to show the number of offspring successfully hatched per female under laboratory conditions is presented in Fig. 2.2. Mean and standard error of trait values for parents and F1 progeny are presented in Table 2.1. The regression analysis for estimating heritability indicated a moderate, significant association between females and F1 progeny in both circum-trunk scales ($b = 0.580$; $t = 3.513$; $P = 0.001$), and ventral scales ($b = 0.568$; $t = 2.510$; $P = 0.014$). The ANOVAs provide further support for these two characters being under genetic control. Both 3 factor ANOVAs showed a significant difference between high and low elevation populations (circum-trunk $F = 8.76$, $P = 0.004$; ventral $F = 8.80$ $P = 0.003$), but not between generations after Bonferroni correction within ANOVA (circum-trunk $F = 4.32$ $P = 0.039$, ventral F = 0.00 P = 0.99). Similarly, both 2 factor ANOVAs on the F1 generation reared under common conditions showed a significant difference between high and low elevation populations (circum-trunk $F = 5.50$, $P = 0.022$; ventral $F = 4.77$ $P = 0.032$) after Bonferroni correction within each ANOVA.

		Mean Low elevation	High elevation	Standard error	Mean difference
Parent	Ventral scale number	60.292	58.996	0.61	1.296
	Circum-trunk scale number	98.718	97.003	0.797	1.715
F1	Ventral scale number	60.065	59.211	0.391	0.854
	Circum-trunk scale number	97.393	96.489	0.386	0.904

Table 2.1. Grand mean, standard error and mean difference between low and high elevation sites for measured scalation traits.

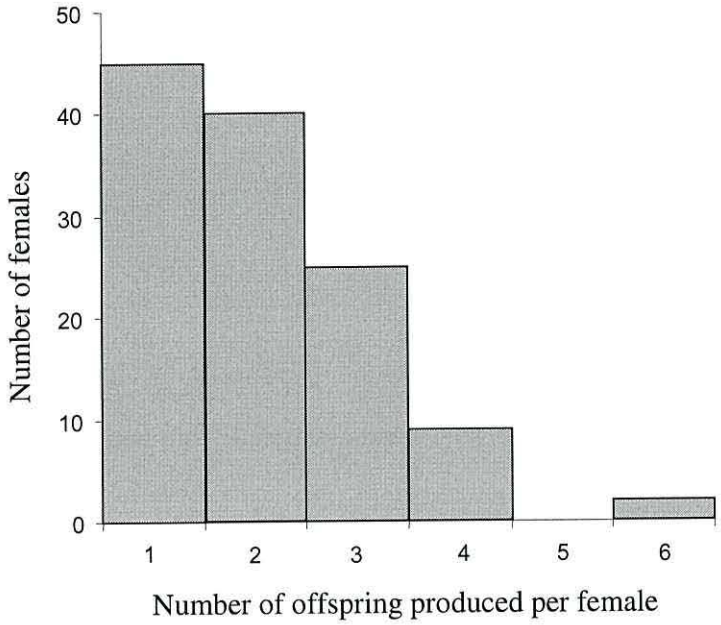

Figure 2.2

Histogram showing number of offspring per female. Data shown excludes one egg per female that was occasionally lost during the transit from field to laboratory.

2.4.2 Phylogenetic analyses

The aligned data set consisted of a 945bp fragment of mtDNA from a total of 97 unique haplotypes (excluding the outgroup). No insertions, deletions or stop codons were found in the data, and the substitutional model of molecular evolution (GTR + I + Gamma) was chosen as the best model to fit the data by MODELTEST 3.0. Stationarity was reached after initial 25% of trees were generated in Bayesian inference, and these 25% were discarded as the "burn-in". Of the 945 sites, 509 were variable, 353 of which were parsimony-informative. Twenty-five unique Puerto Rican and seven unique Dominican haplotypes were identified from those sequenced specifically for this study, (GenBank accession numbers EU599051-75). Seven new mtDNA haplotypes were present in Dominica (GenBank Accession numbers EU599076-82) all of which were unique to the introduced range. The section of the phylogram relevant to this study is presented in Fig. 2.3. Bayesian analysis grouped Dominican haplotypes into two clades within the Puerto Rico phylogeny, the central west Puerto Rican clade and the central east Puerto Rican clade (Fig. 2.3) with high support (posterior probabilities 1.00 and 0.98

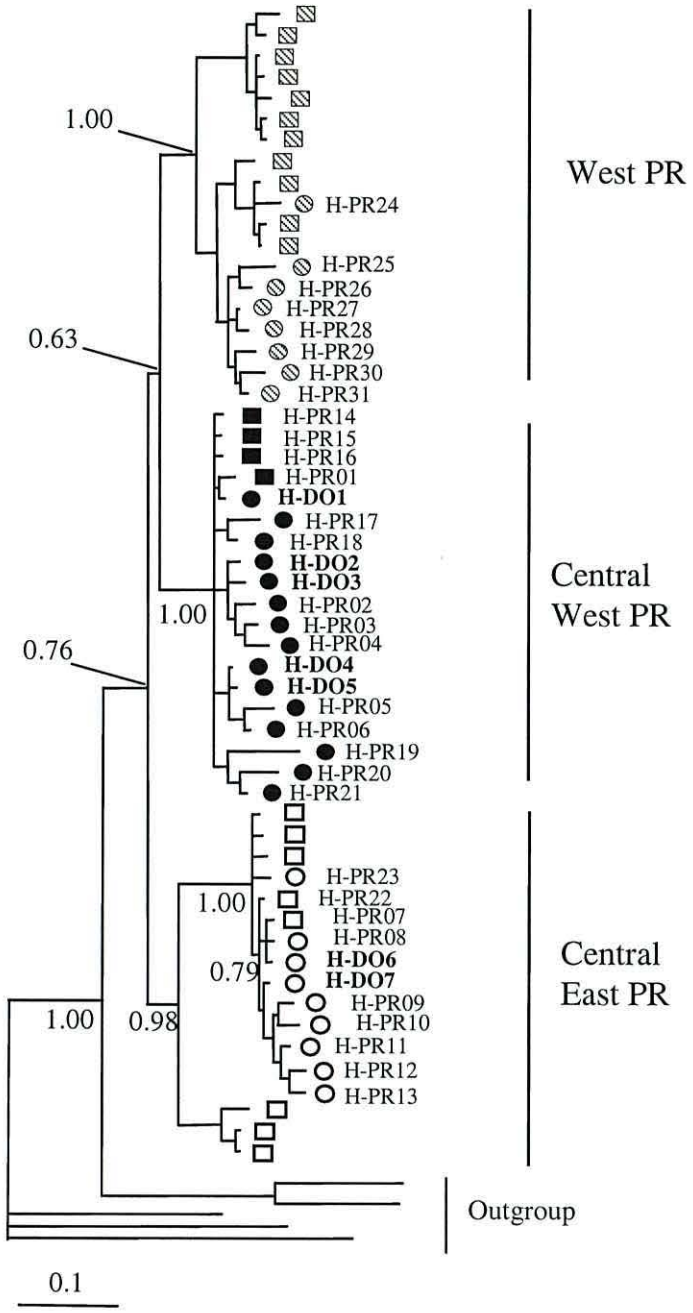

Figure 2.3

Bayesian phylogram of NADH2 sequence data for Puerto Rican and Dominican haplotypes, showing only the section relevant to this study, including the well-supported clades (Central West and Central East Puerto Rico) that contain Dominican haplotypes. Labels H-PR01 to H-PR31 refer to the 25 new haplotypes and six haplotypes (R. Glor, unpublished data) within the Dominican clades. Posterior probabilities are given for major nodes and clades of interest. Symbols are as detailed in the legend for Fig. 2.1.

respectively). Together with sampling locality information (Appendix II), the two clades represent a region in the central north of Puerto Rico which is the likely geographical source of the introduced population (Fig. 2.1). An uncorrected p-distance matrix (constructed using PAUP 4.0b10 [Swofford 1998]) which shows percentage divergence between the seven Dominica haplotypes is presented in Table 2.2. The distribution of haplotypes among sites was not associated with elevation in either Puerto Rico (chi-square 4.112, df = 2, $P > 0.05$; contingency table shown in Table 2.3a), or Dominica (chi-square 3.438, df = 2, $P > 0.05$; contingency table shown in Table 2.3b).

Haplotype	H-D1	H-D2	H-D3	H-D4	H-D5	H-D6	H-D7
H-D1	-	-	-	-	-	-	-
H-D2	0.012	-	-	-	-	-	-
H-D3	0.014	0.011	-	-	-	-	-
H-D4	0.008	0.016	0.018	-	-	-	-
H-D5	0.01	0.017	0.019	0.003	-	-	-
H-D6	0.066	0.069	0.068	0.066	0.069	-	-
H-D7	0.065	0.068	0.067	0.065	0.068	0.005	-

Table 2.2.

Uncorrected p-distance matrix showing the distance between NADH2 haplotypes unique to Dominica.

a)

Haplotype/Clade	Elevation	
	Low (o)	High (o)
H-PR01; H-PR14-21	20	14
H-PR02-04	6	2
H-PR05; H-PR06	8	0

b)

Haplotype/Clade	Elevation	
	Low (o)	High (o)
H-D1	13	11
H-D2; H-D3	13	4
H-D4; H-D5	39	14

Table 2.3

Chi-square test 2x3 contingency table showing observed values for Puerto Rican (a) and Dominican (b) haplotypes

2.4.3 Founder population size

A maximum of 14 alleles were present at two of the five microsatellite loci for Dominican populations (cris 124 and cris 140, [Eales *et al.* 2008]), thus from microsatellite data, the estimated minimum possible effective founder population size was seven. The simulation program which takes into account the allele frequency distribution of source populations was run separately for each locus. The number of individuals for which more than 95% of the 10,000 samples contained a total of at least 14 alleles was 25 for locus cris 124, and 19 individuals for locus 140 (Table 2.4). The number of individuals for which only 5% of the 10,000 samples contained 14 or more alleles was 10 individuals for locus cris 124, and 8 individuals for locus 140 (Table 2.4). The identification of seven mtDNA haplotypes in the introduced Dominican range indicates that the founder population contained a minimum of seven sexually mature females.

Table 2.4

Results of simulation using source population allele frequency distributions for the most variable loci in the source population, cris 124 and cris 140. Values shown are the proportion of the 10,000 samples that contained at least 14 alleles. For both loci, the proportional value for 61-100 individuals sampled was 1 (results not shown). Values in bold type indicate the critical population sizes at which at least 5% of samples, and at which more than 95% of samples, contained at least 14 alleles (smaller and larger values respectively).

Number of individuals sampled	Locus cris 124	cris 128
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0.0018
8	0.0091	0.0233
9	0.0372	**0.0833**
10	**0.0855**	0.1862
11	0.1697	0.3259
12	0.2628	0.4778
13	0.3611	0.6003
14	0.4676	0.7078
15	0.5547	0.7926
16	0.364	0.8542
17	0.7177	0.8997
18	0.7694	0.9292
19	0.8152	**0.9561**
20	0.8591	0.9677
21	0.8958	0.9806
22	0.9218	0.9886
23	0.9416	0.9915
24	0.9489	0.9948
25	**0.9636**	0.998
26	0.9704	0.9975
27	0.9786	0.9984
28	0.9851	0.9994
29	0.9893	0.9994
30	0.9916	0.9995
31	0.9937	0.9999
32	0.9947	0.9997

33	0.9969	0.9998
34	0.9964	0.9999
35	0.9986	1
36	0.999	1
37	0.9991	1
38	0.9995	1
39	0.9995	1
40	0.9999	1
41	0.9997	1
42	0.9999	1
43	0.9999	1
44	0.9999	1
45	0.9999	1
46	0.9999	1
47	0.9999	1
48	1	1
49	1	1
50	1	1
51	1	1
52	1	1
53	0.9999	1
54	1	1
55	1	1
56	1	1
57	1	1
58	1	1
59	1	1
60	1	1

2.5 Discussion

2.5.1 Does phenotypic trait divergence exist between altitudinal extremes of the introduced range, and if so, is there evidence that this trait divergence is heritable?

The common garden experiments showed a strong, repeated, pattern of geographic phenotypic variation with elevation for both parent and F1 progeny in circum-trunk and ventral scale number, which varies in the same direction as the trend documented in the native *A. oculatus* across the same region (Malhotra &Thorpe 1997b). Some degree of genetic control of a trait is required for adaptive responses, and the results of the regression analysis suggest that both measured phenotypic traits have high and significant heritabilities. Moreover, the 2 factor ANOVA of the F1 reared in the common garden also shows altitudinal variation for both characters. Combined, these results indicate that the dominant factor influencing the altitudinal difference in both characters is genetic. However, the 3 factor ANOVA also suggests a role for plasticity in determining ventral scale number. This suggests that *A. cristatellus* is likely to be responding to ecological selective pressure (associated with an altitudinal cline) on both ventral scale and circum-trunk number.

Strong associative evidence for an adaptive response is shown in this study since the variation in an adaptive trait in *A. cristatellus* converges with that shown in native populations (Malhotra & Thorpe 1997a), and is correlated with the same altitudinal cline. The work presented here demonstrates a similar trend to the native *A. oculatus*, although for elevation differences of much smaller magnitude (123m difference between mean of low elevation and mean of high elevation sites for *A. cristatellus* compared to 508m difference between *A. oculatus* sites). Accordingly, the difference between scalation characters of *A. cristatellus* from low and high sites was of a significantly smaller magnitude than recorded for the endemic anole. The decrease in the mean number of scales from high elevations compared to those from low elevations was 1.1% and 1.5% for circum-trunk and ventral scales respectively. Consequently, the more subtle differences in scalation of the invading anole, compared to the endemic anole, may be due both to the more limited time for adaptation, and the more limited altitudinal range of the invader. Selection pressure for scalation characters may be strong enough to produce a rapid evolutionary response in this species because the

predominant environment across much of the lowland range of the introduced species is dry, open xeric woodland, and is also under significant human disturbance. This environmental pressure may have accelerated the spread of the introduced species towards the less disturbed and slightly wetter transitional rainforest environments at higher elevations (which are reached rapidly in the steep topography of the south-western Caribbean coast of Dominica).

2.5.2 Do genotypes in Dominica originate from altitudinally variable sites in the source range, and if so, has geographical segregation of these locally pre-adapted genotypes occurred?

Since *A. cristatellus* have been present on Dominica for less than ten generations, *in situ* historical processes (e.g. genetic drift between Dominican populations) can be excluded as a plausible explanation for the observed geographical phenotypic variation. The genetically-based difference in scale number with elevation is therefore likely to be a result of, either pre-adapted haplotype assortment, or natural selection. Results from the phylogenetic analysis indicate that the geographical origin for the introduced haplotypes is in the central north of Puerto Rico. This region covers a range of elevations between 0-500m above sea level. Segregation of mtDNA haplotypes by elevation was not supported by the chi-square analysis for either Puerto Rico or Dominica, suggesting that any pattern of altitudinal phenotypic variation within Dominica cannot be directly attributed to altitudinal haplotype segregation. The remaining cause of the altitudinal variation in ventral scale number is response to ecological selection pressure that varies with elevation in Dominica. It is possible that maternal effects are responsible for a proportion of the heritability and altitudinal variation in F1 progeny in the phenotypic traits; however, raising F2 progeny to eliminate maternal effects significantly increases the time costs and logistical complexity of vertebrate common garden experiments.

2.5.3 Are the estimates of founder population genetic diversity consistent with the genetically-based trait variation in the introduced population?

Since the altitudinal variation in *A. cristatellus* scale number has been shown in this study to have a genetic basis, it follows that sufficient genetic diversity must exist within Dominican populations to enable this trait to respond to ecological selection

pressure. Uncorrected divergence values of up to 6.9% between Dominican haplotypes are consistent with microsatellite data. Analyses of microsatellite data indicate a weak genetic founder effect within Dominica (Eales *et al.* 2008a). Simulations based on allele frequency distributions of the most variable microsatellite loci suggest that the effective founder population size in Dominica was moderate (in the region of 19-25 individuals), and are consistent with a minimum estimate of seven females from mtDNA haplotype data.

This study revealed that female *A. cristatellus* can store sperm for up to 84 days after the most recent mating, and produce up to six viable offspring under isolated laboratory conditions (Fig. 2.2). Consequently, each sexually active female transported from the source range can potentially introduce up to six paternal genotypes to introduced populations. Based on the presence of at least seven sexually mature females in the initially transported founding population, sperm storage and multiple paternity could be responsible for the introduction of up to 49 genotypes in Dominica. Sperm storage is a well-recognised phenomenon in lizards (Cuellar 1966; Sever & Hamlett 2002), and together with multiple mating by females, documented in *Anolis* by Tokarz (1998) has significant implications for the potential genetic contribution by female *Anolis* that have been reproductively active in the source range prior to introduction. A study by Johnson *et al.* (in review) found that 52% of *A. cristatellus* females produced progeny fathered by more than one male, and for *A. sagrei* the figure was 81% (Calsbeek *et al.* 2007). Female *Anolis* are able to produce viable offspring from sperm that had been stored from between 107 days in *A. sagrei* (Calsbeek *et al.* 2007) and 10 months in *A. carolinensis* (Passek 2002). The capacity for the introduction of multiple paternal genotypes by sexually active females in the initially transported population may result in a much higher initial effective population size for *A. cristatellus* than the actual number transported. Thus, the reproductive ecology of *Anolis* may represent an important mechanism by which introduced populations might minimise the extent of genetic founder effects resulting from demographic bottlenecks, and increase the capacity for the introduced population to present adaptive responses to environmental variability based on genetically controlled traits.

In this study, we demonstrate phenotypic variation in adaptive traits that vary with geographical clines within the introduced range of an invasive population of *Anolis*

cristatellus. This work suggests that variation is likely to result from both adaptive, genetically based responses to ecological selective pressure, and via phenotypic plasticity. The work presented here provides further evidence of rapid evolution in *Anolis* phenotypic traits, as documented for the native Dominican anole (Malhotra & Thorpe 1991a; Thorpe *et al.* 2005). Our results provide direct evidence that the strength of selective processes can result in matching of phenotype to ecological conditions within around ten generations of exposure to such pressures. MtDNA evidence and estimates of founder population size support previous work that demonstrates a weak founder effect in Dominican *A. cristatellus*, and is consistent with the genetic basis of trait variation. It is also suggested that the genetic capacity to respond to selective pressure may partly result from sperm storage and multiple paternity in *Anolis*.

This study addresses key questions concerning the interactions between ecological and evolutionary processes during the early stages of invasions. The observation of a rapid adaptive response within *c.* ten generations in populations that have retained significant genetic diversity from source populations suggests that a similar response is possible in other recent introductions. This knowledge is vital for the implementation of appropriate management strategies in the conservation issues raised by introduced species. This study demonstrates the value of linking molecular analyses with experimental common garden studies, to provide an explanation behind the observed variation in introduced species phenotype. It is shown that an appreciation of both introduction history and life history characteristics is essential to elucidate the processes which may facilitate adaptive genetic responses in introduced populations, and therefore predict their invasive ability.

CHAPTER 3

Weak founder effect signal in a recent introduction of Caribbean *Anolis*

3.1 Abstract

Species introductions provide a rare opportunity to study rapid evolutionary and genetic processes in natural systems, often under novel environmental pressures. Few empirical studies have been able to characterise genetic founder effects associated with demographic bottlenecks at the earliest stages of species introductions. This study utilises prior mtDNA information which identifies the putative source population for a recently established (*c.* seven years between import and sampling) species introduction. Evidence for a founder effect is investigated in a highly successful introduction of a Puerto Rican *Anolis* species that has established itself on Dominica to the localised exclusion of the native, endemic anole. Five highly polymorphic microsatellite loci were used to explore the partitioning of genetic diversity within and between native source, native non-source, and introduced populations of *Anolis cristatellus*. Group comparisons reveal significantly lower allelic richness and H_E in introduced populations compared to native populations, however, tests for heterozygosity excess relative to allelic richness failed to provide consistent evidence for a founder effect within introduced populations. Significant levels of within-population genetic variation were present in both native and introduced populations. This study suggests that aspects of the reproductive ecology of *Anolis* (high fecundity, sperm storage and multiple paternity) offer an important mechanism by which genetic variation may be maintained following demographic bottlenecks and founder events in some squamate taxa.

3.2 Introduction

Introduced species have long been recognised as having important economic and ecological effects (Elton 1958; Simberloff & Stiling 1996; Pimentel 2000; Sakai *et al.* 2001) and as such they have been much studied. More recently, their potential as model systems of evolutionary processes has been realised (Lambrinos 2004). The introduction of a small founder population into a new, non-native locale (often by anthropogenic means of transportation) offers a rare opportunity to study rapid evolutionary processes under something approaching natural conditions. Furthermore, because introductions often involve novel environments with different ecological challenges to those which the species has been previously exposed, they have been frequently associated with adaptive evolution (Rasner *et al.* 2004). Molecular genetic methods are a powerful tool in invasion studies, providing evidence to link introduced populations to their potential native range sources as well as exposing genetic differences between these areas, suggesting that evolutionary processes may have taken place (Cornuet & Luikart 1996).

Identifying the source population is a significant step in the investigation of an invasion event, enabling reconstruction of past events leading up to the introduction (Williamson 1996) and exploration of the potential for appropriate control and management. Molecular data (e.g. mtDNA sequence data) together with phylogeographic analysis may be used to elucidate the most probable source population in the native range (Hufbauer *et al.* 2004; Eales *et al.* 2008b). Subsequent morphological and genetic comparison of the source and invasive populations using fine scale nuclear markers (e.g. microsatellites) can provide detailed information about the amount of genetic variation introduced (Cornuet & Luikart 1996), the effects of genetic diversity on introduction success (Sakai *et al.* 1996), population fitness (Reed & Frankham 2003) and species ecology (Tsutsui *et al.* 2000; Tsutsui *et al.* 2001). In addition, the study of processes associated with known small founder populations and genetic bottlenecks provides an invaluable model that has relevance to the substantial reductions in population size faced by many endangered species (Frankham *et al.* 2002; Jehle & Arntzen 2002; Moran 2002; Smith & Wayne 1996) and to the understanding of natural island radiations.

The founder effect was first described by Mayr (1963) as the establishment of a new population by a small number of individuals that derived from a larger source population. The founder population typically carries a small proportion of the genetic variation contained within the original source population. This sampling effect can result in the introduced founder population having distinct genetic and associated phenotypic characteristics which may differ from those contained in the source population. Genetic founder effects have been documented in a number of empirical examples (Baker & Moeed 1987; Hawley *et al.* 2006). However, some recent studies have failed to detect unequivocal genetic evidence for founder effects in introduced populations (Zenger *et al.* 2003; Durka *et al.* 2005). Several possibilities may explain these inconsistencies. A time lag between introduction and genetic characterisation presents an opportunity for new mutations to reduce the genetic founder effect in introduced populations. However, assuming standard mutation rates (Goldstein & Schlötterer 1999), and the short time lags of some studies corresponding to less than 20 generations (Astanei *et al.* 2005; Busch *et al.* 2007), mutation is unlikely to be a significant source of founder population genetic variation. A greater effect on diversity levels is likely through another mechanism that increases within-population genetic variation i.e., subsequent introductions of haplotypes not previously introduced to the newly occupied range. Multiple introductions from genetically distinct areas of a source range could feasibly reduce or erase the genetic signature of a founder effect via gene flow (Kolbe *et al.* 2004).

The amount of genetic diversity present in an introduced population is directly related to the amount provided from the source population (Veltman *et al.* 1996). A large founding population is likely to carry a significant amount of genetic diversity, and, as described above, additional variation can be provided by multiple introductions (Astanei *et al.* 2005). A full record of introduction history together with thorough genetic characterisation of both native range and introduced populations is rare in empirical studies, but is a necessary component for the full understanding of population genetic processes during introductions. Past analyses have often focused on characterising the genetic structure of introduced populations, and the comparison of both native range and introduced populations is less common (although see Novak & Mack 1993; Maron *et al.* 2004; Durka *et al.* 2005). Many recent studies have focused on successful introductions that have passed through the initial phases of colonisation, establishment

and spread, despite the increasing global incidence of human-mediated species introductions that have been observed and documented within decades of initial import (Facon *et al.* 2003; Shigesada & Kawasaki 1997; Williams 1969). Such studies have minimal application to issues of concern in invasion studies, the potential for future establishment and spread of the non-native population, and the relationship between this and the extent of genetic founder effects in introduced populations.

Given the increasingly recognised ecological and economic importance of species introductions, empirical studies are urgently required to elucidate the effects of demographic bottlenecks on the genetic characteristics of early stage introduced populations, and the associated impacts on invasion success (Spencer *et al.* 2000). The discovery of the non-native *Anolis cristatellus* (Sauria: Iguanidae) inadvertantly imported onto the Lesser Antillean island of Dominica within seven years of the onset of this study, has provided just such an opportunity. Anoles have been widely transported throughout the Caribbean (Losos *et al.* 1993) both by natural dispersal mechanisms such as hurricanes (Calsbeek & Smith 2003; Stenson *et al.* 2004) and through anthropogenic transportation (Campbell 1996). However, the Dominica discovery is rare in that the single point and timing of import is recent and known, its range within the island has been well-documented since an early stage, and the anole niche is filled with a well-studied native competitor, *A. oculatus* (Malhotra & Thorpe 1991; Malhotra & Thorpe 1997; Malhotra & Thorpe 2000; Stenson *et al.* 2004).

The nature of *Anolis* community formation in the Caribbean exposes the vulnerability of Lesser Antillean islands to invasion by anoles originating from the Greater Antilles. Lesser Antillean anoles generally experience low levels of competition, due to islands typically containing no more than two species that occupy different microhabitats and niches. Greater Antillean multi-species assemblages with up to eleven species occurring in sympatry on a single island (Losos & Thorpe 2004), have evolved under stronger inter-specific interactions to become relatively more competitively robust (Losos & De Queiroz 1997). Greater Antillean anoles may therefore have a strong competitive advantage when arriving on Lesser Antillean islands, a situation which has only recently been documented (Greene *et al.* 2002; Malhotra *et al.* 2007), whilst invasions among islands within the Greater Antilles and among islands within the Lesser Antilles are more common (Fitch *et al.* 1989, Losos *et al.* 1993). The main import dock on

Dominica, as on other Caribbean islands, routinely receives containers from across the Caribbean basin and mainland South America, thus there are numerous opportunities for cross-island importation of inadvertent stowaways. *A. cristatellus* was first identified on Dominica adjacent to the import dock at Deepwater Harbour in 2000, whilst a survey of the island in 1998 had found no evidence of the alien species (Thorpe, pers. comm.). Since importation, the introduced anole has spread approximately 6 km north and 8 km south of the dock along the west coast of the island and has penetrated inland areas within this range up to 200 m altitude. On its native Puerto Rico (Greater Antilles) *A. cristatellus* is the most abundant anole of the 13 species naturally occurring on the island, and occupies a wide range of habitats from sea level to over 700m altitude (Eales, pers. obs.).

Phylogenetic analysis using mtDNA data has identified a putative source area in central Puerto Rico for Dominican *A. cristatellus* (Eales *et al.* 2008b), which is combined here with Puerto Rican non-source populations and a broad geographic sampling strategy across the introduced range to elucidate spatial population genetic characteristics. This approach extends the scope of this study as a model of species introductions, enabling genetic comparisons between populations of varying introductory history (native source, native non-source and introduced). Using the unpublished phylogenetic information about the geographical location of putative source populations, this study tests for a genetic founder effect in Dominican *A. cristatellus* by first comparing levels of genetic diversity among native and introduced populations. Second, within-population parameters are used to test for a genetic founder effect in introduced populations, as compared to native range populations. Finally, measures of population genetic structure are used to determine the partitioning of genetic diversity across native and introduced populations.

3.3 Materials and methods

3.3.1 Sample collection

Tail tip biopsies, preserved in 95% ethanol, were obtained from *Anolis cristatellus* lizards in Dominica, Lesser Antilles (introduced range) and Puerto Rico, Greater

Antilles (native range). Nine local populations were sampled from Dominica, at five low altitude sites, between 3-41m above sea level, and four high altitude sites, between 128-176m above sea level (Fig. 3.1). Phylogenetic analysis (Eales *et al.* 2008b *submitted*) based on sequencing of a 945 bp fragment of mitochondrial NADH2 identified seven founding haplotypes present in the introduced Dominican population (GenBank accession numbers EU599076-82, Appendix III), which were closely related to populations from central Puerto Rico. The present study includes three sample localities from the putative source area in central Puerto Rico and four sample localities in Puerto Rico from outside of the putative source range (Fig. 3.1). At each locality, 47 tail tips were collected from individual *A. cristatellus*. Population site locations were recorded using a handheld Garmin 60CS GPS, and postitions are given in Appendix I.

3.3.2 DNA extraction and amplification

Genomic DNA was extracted from tail tips using the Chelex extraction method (Walsh *et al.* 1991). Initially, six polymorphic loci were used in this study, developed as molecular markers specifically for *A. cristatellus* (Glor *et al.* 2007). None of the loci used were sex linked. Multiplexed polymerase chain reaction (PCR) amplification was carried out using a reaction volume of 5 µL containing 10 ng of template DNA, 2.5µl Qiagen Multiplex PCR Master Mix Q-Solution (Qiagen, Hilden, Germany), 1 pmol of forward and reverse primers CRIS 22, CRIS 124, CRIS 128, CRIS 136 and CRIS 140 and 1.5 pmol of CRIS 92 (Glor *et al.* 2007). Forward primers were fluorescently labelled. The thermocycling regime consisted of an initial denaturation step at 95°C for 15 minutes, followed by 25 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 90 seconds and extension at 72°C for 60 seconds, followed by a final extension phase at 60°C for 60 seconds. Labelled fragments were electrophoresed on an ABI 3130 Genetic Analyser (Applied Biosystems) with GeneScan™ 600 LIZ® (Applied Biosystems) as internal size standard. GENEMAPPER v 3.0 (Applied Biosystems) was used to estimate allele sizes using automatically generated bins, and bin calls were visually confirmed and double checked.

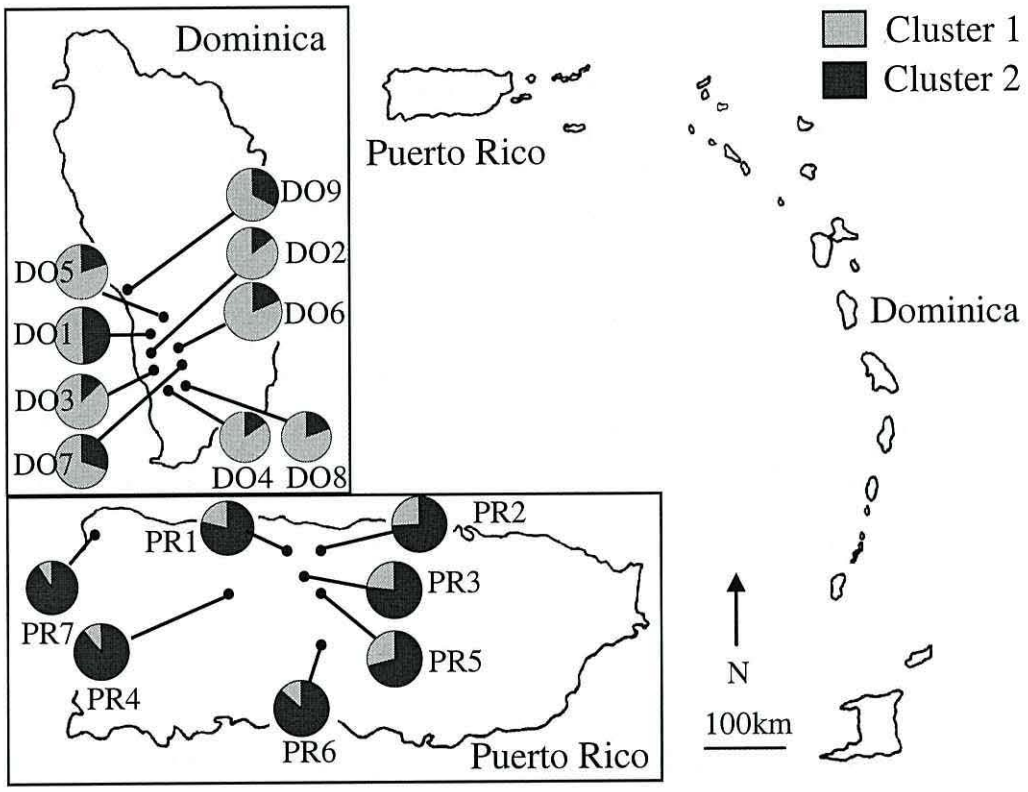

Figure 3.1 .

Location of sampling sites for *A. cristatellus* on Puerto Rico and Dominica. Sample localities representing the putative source area on Puerto Rico of the invasive Dominican *A. cristatellus* are marked PR1-3. Localities PR4-7 represent non-source population on Puerto Rico. Pie graphs indicate the proportion of individuals' genomes from each population assigned to each of two clusters identified by STRUCTURE (see text for further details).

3.3.3 Preliminary data analysis

Only individuals with data for all loci were included in the analyses. Allele frequency data is given in Appendix IV. The data set comprised 527 genotyped *Anolis* from seven native Puerto Rican (three source, four non-source) and nine Dominican (introduced) populations. Conformance to Hardy-Weinberg equilibrium (HWE) was assessed with exact tests implemented in GENEPOP on the Web [http://wbiomed.curtin.edu.au/genepop/genepop_op6.html] (Raymond & Rousset 1995), which uses a Markov chain method to estimate significance. GENEPOP was also used to

test for population-specific departures from linkage disequilibrium between pairs of loci.

3.3.4 Among-population genetic differentiation

Five population-level measures of genetic diversity were calculated in FSTAT (Goudet 2001): allelic richness, the number of alleles per locus weighted by sample size; H_E expected heterozygosity as measured by Nei's gene diversity (Nei 1973); H_O observed heterozygosity per locus; F_{ST}, the level of genetic differentiation within populations (Weir & Cockerham 1984) and Nei's F_{IS}, the divergence of observed heterozygosity from expected heterozygosity per locus. To test for differences between native range and introduced populations in these parameters, one-sided group comparisons in FSTAT with 1000 permutations were used.

3.3.5 Within-population genetic diversity

Within-population tests for genetic founder effects rely on the expectation that rare alleles are lost rapidly during a population bottleneck and measure this rare allele deficiency relative to other allele parameters to detect a significant effect relative to pre-population bottleneck proportions (Maruyama & Fuerst 1985; Luikart & Cornuet 1998). Two measures were used in this study to detect a within-population genetic signal from a founder event. First, following the expectation that many rare alleles are lost following a population bottleneck, we test for a significant change in allele frequencies at microsatellite loci. Populations at mutation-drift equilibrium are expected to have a large proportion of alleles at low frequency (Luikart *et al.* 1998), typified by an L-shaped allele frequency distribution. Populations that have recently undergone founder events are expected to show a mode-shift distortion in allele frequency distributions to the effect that low frequency alleles become less abundant, resulting in a more even distribution of allele frequencies. BOTTLENECK v 1.2.02 (Cornuet & Luikart 1997) was used to generate the distribution of observed alleles in each population to detect a mode-shift distortion away from the typical L-shaped distribution. Second, because rare alleles are lost more rapidly than overall heterozygosity during a founder event (Nei *et al.*, 1975), bottlenecked populations are likely to have higher observed heterozygosities than

expected relative to the total number of alleles available (Cornuet & Luikart 1996). Because this effect is most apparent immediately following a population bottleneck, the use of apparent heterozygote excess to detect a population bottleneck may be limited to severe or very recent founder events (Cornuet & Luikart 1996). Heterozygosity excess (relative to the number of alleles detected in each population) was tested in BOTTLENECK under all three proposed models of microsatellite mutation (the infinite allele model, IAM, the stepwise-mutation model, SMM, and the two-phased model, TPM (70% SMM and 30% variable), to compare the sensitivity of each. Statistical significances of the results from each model were tested using the Wilcoxon test.

3.3.6 Population genetic structure

To compare genetic structure within and between pairs of populations in the native and introduced range, pairwise F_{ST} (Weir & Cockerham 1984) was calculated between pairs of native range and introduced populations, and between pairs of populations within these population history groupings. Significance of multiple pairwise comparisons was determined using the strict Bonferroni method and the significance level was adjusted to 0.00042 for 120 comparisons. Population F_{ST} and significance value matrices were calculated by FSTAT across 15,000 permutations.

Analyses of molecular variance (AMOVAs) (Excoffier *et al.* 1992) were performed using ARLEQUIN v 3.01 (Excoffier *et al.* 2005), to investigate the distribution of genetic variation among individuals, populations and islands. ARLEQUIN uses both allelic content and frequencies of haplotypes to estimate genetic structure and significance is tested with non-parametric permutations, in this study 20,000 permutations were used. Separate island AMOVAs were performed for Puerto Rico and Dominica to investigate partitioning of genetic variation within islands. Then, two hierarchical AMOVAs were run to examine the partitioning of genetic variation between islands. First, between Puerto Rican source populations and Dominica, and second, between all Puerto Rican populations and Dominica. The software STRUCTURE v 2.1 (Pritchard *et al.* 2000) uses a Bayesian Markov chain Monte Carlo (MCMC) approach to cluster individuals into population groupings whilst minimising Hardy-Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. The program is most often used to

estimate the most likely number of populations based on partitioning of genetic variation, and to determine the average percentage membership of each population to these hypothetical clusters. In this study, STRUCTURE was primarily used to indicate the strength of genetic partitioning between Puerto Rican and Dominican populations, indicated by the proportion of populations assigned to each of two user-defined clusters (i.e. when K=2). We also used STRUCTURE in the conventional practice to determine the most likely number of clusters the populations may be partitioned into. STRUCTURE was run for values of K ranging from 1-6 with initial burn-in of 10,000 MCMC iterations and a data collection period of 10^6 iterations. Ten independent simulations were run for each value of K and returned consistent results. No prior information was used on the population of origin of each individual. The admixture model was used, in which individuals may have mixed ancestry and the correlations model, which takes into account that closely related populations might have correlated allele frequencies. A principal component analysis (PCA) was performed on populations using PCAGEN [http://www2.unil.ch/popgen/softwares/pcagen.htm], which ordinates populations in multidimensional space using allele frequency data.

3.4 Results

3.4.1 Preliminary analysis

All six loci were polymorphic and in linkage equilibrium in each of the populations sampled. Five of the six loci were in HWE, after correcting for multiple comparisons. Fifteen of the 16 sampled populations were heterozygote deficient at locus CRIS 92, thus allele data from this locus were excluded from the subsequent analysis. Departures from HWE in CRIS 92 are unlikely to represent a Wahlund effect (population genetic substructure within predefined populations) because the departures were consistently associated with one locus and not across loci for populations. Because population sampling localities were restricted to 200 m^2 of continuous *Anolis* habitat, and home range size for an individual anole has been estimated up to 34 m^2 (Schoener & Schoener 1982), departures from HWE are unlikely to represent population genetic substructure within the limited sampling areas in this study. A more likely cause of the heterozygote deficiency in CRIS 92 is the presence of null alleles, which can result from the

preferential amplification of small alleles causing large allele dropout or mutations in the primer annealing site for an allele. Other causes of the heterozygote deficit may be close linkage of CRIS 92 to loci under selection or slippage during PCR amplification. Significant departures from HWE were observed in four of the 80 single-locus exact tests for heterozygote deficit in the remaining five loci following sequential Bonferroni correction (Rice 1989). The distribution of these deviations was spread across three loci and four populations and therefore are unlikely to represent any consistent pattern of departure from HWE with either locus or population.

3.4.2 Among-population genetic differentiation

Figure 3.2 shows pooled allele frequencies per locus for Puerto Rican (PR) non-source (4), PR source (3) and introduced Dominican (9) populations. At each of the five loci, and overall, introduced populations had fewer alleles (47) than either non-source (76) or source (68) populations, despite a consistently greater sample size for introduced populations. Three alleles across three different loci were present only in the Dominican populations, in eight, seven and four of the nine introduced populations respectively. Two alleles were present in PR source and Dominican populations only, and one allele was present in PR non-source and Dominican populations but not PR source populations. One-sided group comparisons in FSTAT showed significant differences between all PR and Dominican populations in two of the five measures of genetic diversity; allelic richness and H_E (Table 3.1) with PR populations showing greater levels of diversity for both measures. Group comparisons between PR source and Dominican populations showed a significant difference in H_E only, with PR source populations showing more expected heterozygosity.

Island / Population History	*Npop*	*A*	H_O	H_E	F_{IS}	F_{ST}
A						
Puerto Rico	7	9.938	0.798	0.832	0.040	0.043
Dominica	9	8.905	0.774	0.798	0.031	0.033
P-value		0.018	0.123	0.017	0.335	0.215
B						
Puerto Rico (source)	3	9.934	0.802	0.837	0.042	0.025
Dominica	9	8.905	0.774	0.798	0.031	0.033
P-value		0.070	0.166	0.032	0.372	0.624

Table 3.1.

A comparison of some population parameters between all Puerto Rican populations of *Anolis cristatellus* and the introduced Dominican population (A) and between three populations in PR (identified as the source of the invading population by mtDNA analysis), and the introduced Dominican population (B). Number of populations is given in column *Npop*. Parameters include allelic richness (*A*), observed heterozygosity (H_O), expected heterozygosity (H_E), the level of genetic differentiation (F_{ST}), and deviations from HWE (F_{IS}). One-sided probabilities for group comparisons calculated using FSTAT.

3.4.3 Within-population genetic diversity

There were large differences in results of tests for apparent heterozygosity excess depending on the underlying mutation model employed. Under the IAM, all PR and six of the nine introduced populations showed significant apparent heterozygote excess (Table 3.2). With an underlying SMM, no population showed significant apparent heterozygosity excess, and under the TPM, one PR source and two introduced populations showed significant apparent heterozygosity excess expected with a population bottleneck: PR3, DO5 and DO6. The IAM is reported to be better able to detect subtle population bottlenecks, although it has also been known to identify them in non-bottlenecked populations (Luikart & Cornuet 1998). Although allele frequency graphs show a reduction in the number of alleles present in introduced populations (Fig. 3.2), allele frequencies followed an L-shaped distribution, and there was no evidence

Population / History	Heterozygosity excess *P* values		
	IAM	SMM	TPM
Puerto Rico (source)			
PR1	0.016	NS	NS
PR2	0.016	NS	NS
PR3	0.016	NS	0.016
Puerto Rico (non-source)			
PR4	0.031	NS	NS
PR5	0.016	NS	NS
PR6	0.016	NS	NS
PR7	0.016	NS	NS
Dominica (introduced)			
DO1	0.016	NS	0.016
DO2	0.016	NS	0.016
DO3	0.031	NS	NS
DO4	NS	NS	NS
DO5	0.016	NS	NS
DO6	0.016	NS	NS
DO7	NS	NS	NS
DO8	NS	NS	NS
DO9	0.016	NS	NS

Table 3.2.

Tests of within-population heterozygosity excess with three underlying mutational models (IAM, SMM and TPM) performed using BOTTLENECK. Probability values were determined using one-tailed Wilcoxon tests.

of a mode shift in allele frequencies towards intermediate values as may be expected in a population that has undergone sequential or prolonged bottlenecks.

3.4.4 Population genetic structure

Significant differences in pairwise F_{ST} values exist between all population pairs, (Table 3.3) except between eight pairs of introduced populations and one pair of Puerto Rican populations (PR3 and PR5). Values showed typically low amounts of genetic differentiation, ranging from 0.014 to 0.103. Mean pairwise F_{ST} for between-island pairs was 0.058, slightly higher than the mean for Puerto Rican population pairs (0.045) and the mean for Dominican population pairs (0.036). Separate island AMOVAs revealed that limited amounts of geographical structure exist within both Puerto Rico and Dominica, with 4.4% and 3.3% of total genetic variation residing among populations on the islands, respectively, and the remaining 95.6% and 96.7% residing within populations (Table 3.4). Evidence of high within-population variation is also provided by the results of the hierarchical AMOVA test, which examines the partitioning of genetic variation within and between Puerto Rico and Dominica. This test revealed that the majority of total genetic variation (94.2%) resided within populations, 3.7% resided among populations within islands and 2.1% was due to differences between islands (Table 3.4). Hierarchical AMOVA indicated that slightly less variation is partitioned between the PR source range (three populations) and Dominica, than between Puerto Rico (seven populations) and Dominica (1.6% and 2.1%, Table 3.4).

The highest likelihood for the number of genetic clusters identified by STRUCTURE was returned for K=2. Over half the number of individuals within each of the 16 populations were assigned by STRUCTURE to one of the two user-defined clusters representing Puerto Rico and Dominica (Fig. 3.1). Moreover, more than 70% of individuals' genomes were correctly assigned to the clusters representing the two islands, except for DO1 (51%) and DO9 (67%), demonstrating that despite a low degree of genetic variation between islands (AMOVA), some genetic differentiation exists between Puerto Rico and Dominica. The PCAGEN analysis on allele frequency data provided results concordant with the STRUCTURE analysis.

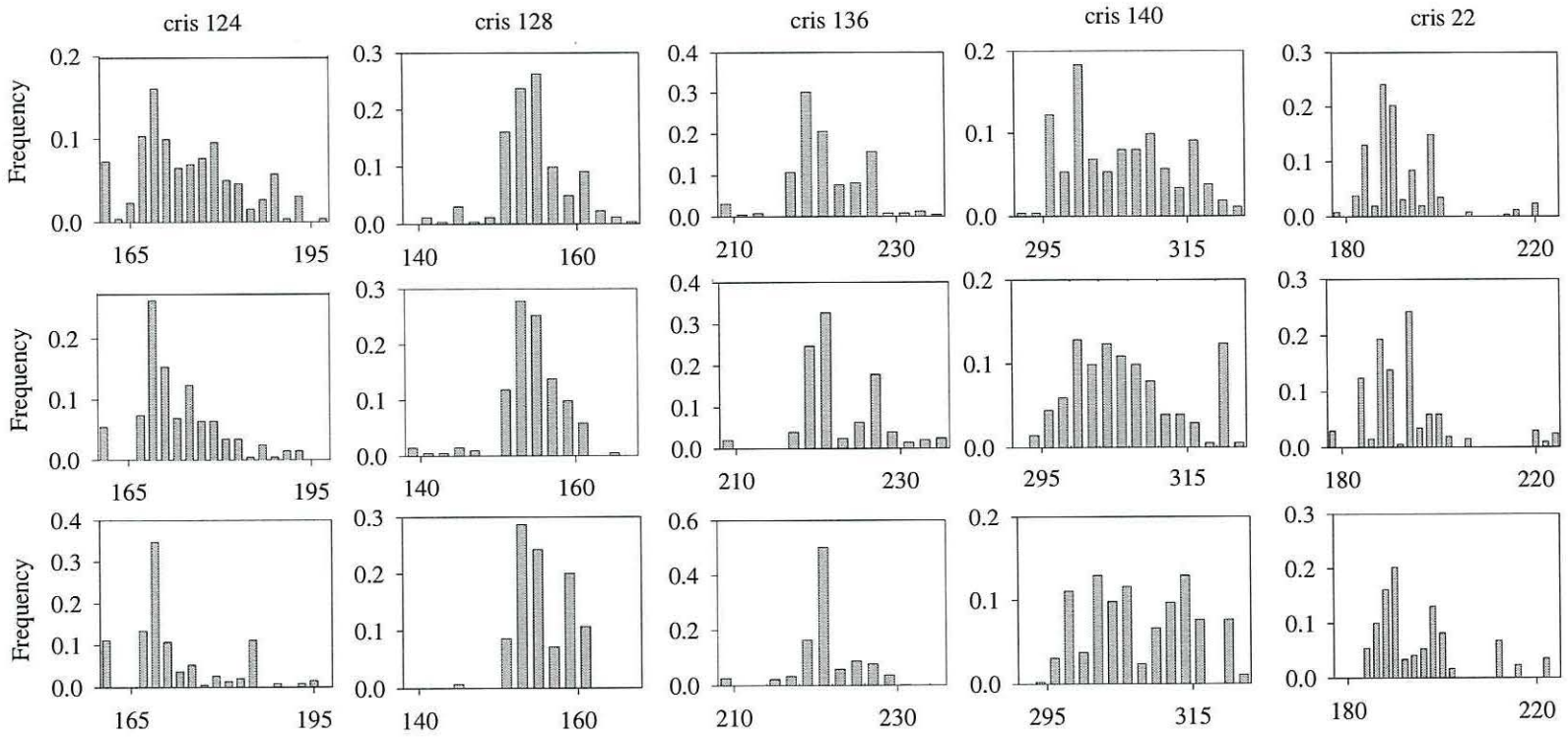

Figure 3.2.
Pooled microsatellite allele frequency histograms by locus. First row shows native non-source Puerto Rican populations (PR4-7), second row shows source Puerto Rican populations (PR1-3), third row shows introduced Dominican populations (DO1-9).

	Puerto Rico Non-source			Source				Dominica Introduced populations								
	AB	CI	OR	TU	CB	LT	N	BH	BL	GH	GL	MH	ML	RH	RL	J
AB	-	0.0401*	0.0271*	0.0792*	0.0567*	0.0449*	0.0212*	0.0659*	0.0917*	0.0884*	0.0846*	0.0647*	0.0598*	0.0928*	0.041*	0.0539*
CI		-	0.0612*	0.0711*	0.0404*	0.0323*	0.0157	0.0479*	0.0671*	0.044*	0.0444*	0.028*	0.0355*	0.0596*	0.0279*	0.0323*
OR			-	0.0581*	0.063*	0.0556*	0.0255*	0.0758*	0.0917*	0.093*	0.103*	0.0657*	0.0582*	0.0833*	0.0649*	0.0606*
TU				-	0.0504*	0.0445*	0.0439*	0.0728*	0.0974*	0.0896*	0.0972*	0.0878*	0.0796*	0.0768*	0.0844*	0.0625*
CB					-	0.0277*	0.0273*	0.041*	0.0835*	0.0638*	0.0652*	0.0476*	0.0505*	0.06*	0.0431*	0.0354*
LT						-	0.0188*	0.0174*	0.0472*	0.0365*	0.0379*	0.0387*	0.0362*	0.031*	0.0384*	0.0219*
N							-	0.0391*	0.0584*	0.0506*	0.0515*	0.0304*	0.0335*	0.058*	0.0311*	0.0296*
BH								-	0.0382*	0.025	0.0323*	0.0297*	0.029*	0.0365*	0.0462*	0.0218
BL									-	0.0451*	0.0562*	0.0365*	0.0213*	0.0627*	0.0515*	0.0411*
GH										-	0.0075	0.0078	0.0205	0.0166*	0.0564*	0.019
GL											-	0.0179*	0.0268*	0.0261*	0.0579*	0.0157*
MH												-	0.0047	0.0301*	0.0389*	0.0096
ML													-	0.0298*	0.0254*	0.0142*
RH														-	0.0613*	0.0316*
RL															-	0.0387*
J																-

Table 3.3.
Pairwise *F*ST values * denotes significance at 0.05 level.

Range/ Source of Variation	df	Variance components	Percentage of Variation	*P* value
Puerto Rico				
Among populations	6	0.095	4.4	0.000
Within populations	457	2.078	95.6	0.000
Dominica				
Among populations	8	0.069	3.3	0.000
Within populations	581	1.995	96.7	0.000
PR (source) and Dominica				
Among groups	1	0.033	1.6	0.007
Among populations within groups	10	0.066	3.1	0.000
Within populations	780	2.020	95.3	0.000
Puerto Rico and Dominica				
Among groups	1	0.045	2.1	0.000
Among populations within groups	14	0.080	3.7	0.000
Within populations	1038	2.030	94.2	0.000

Table 3.4.

Analyses of molecular variance (AMOVAs) showing distribution of genetic variation among islands, populations and individuals for separate island and hierarchical population groups.

When plotted in two-dimensional space, a clear separation was revealed along the PCA1 axis between Puerto Rican and Dominican populations, except DO1 (Dominica) which groups with Puerto Rican populations, Fig. 3.3. PCA 1 represents 28.87% of variation in the data and PCA 2 represents 15.69% of variation in the data. These relatively low PCA axis weightings are concordant with a limited degree of between-group or population structuring and the high amounts of within-population genetic variation revealed by the AMOVAs.

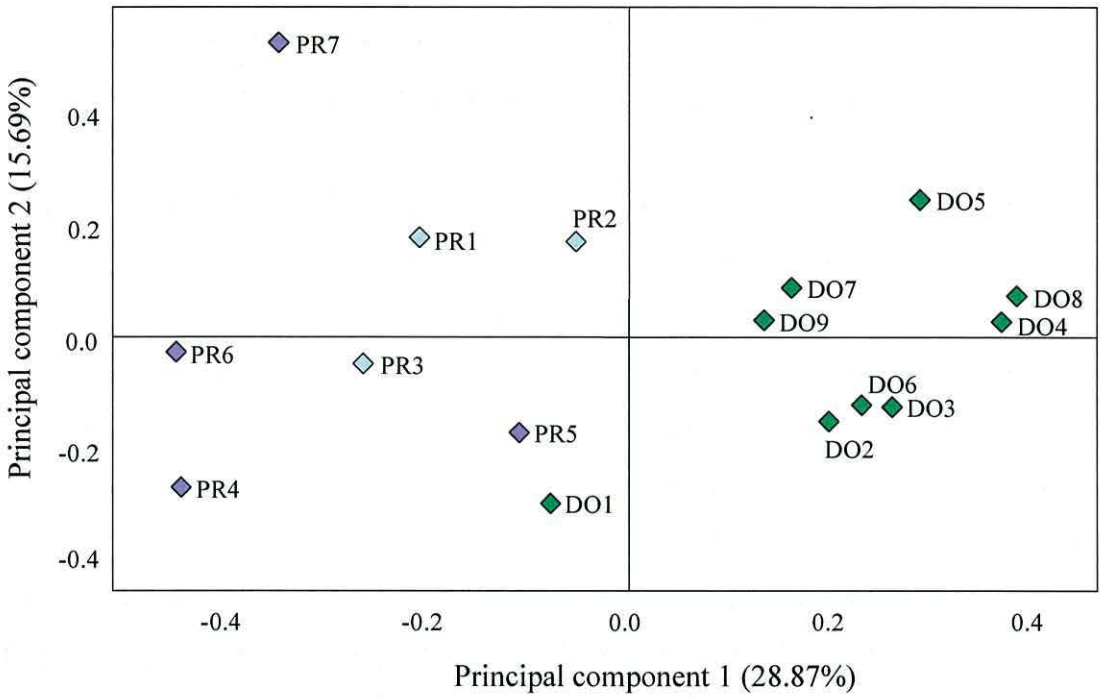

Figure 3.3

Principal component analysis scatter diagram based on allele frequency of 16 *A. cristatellus* populations from Puerto Rico and Dominica. Putative source Puerto Rican populations (PR1-3, shown in blue); native non-source Puerto Rican populations (PR4-7, shown in pink); introduced Dominican populations (DO1-9, shown in green).

3.5 Discussion

The general overview from the analyses is that there is limited genetic evidence of a population bottleneck in introduced Dominican populations of *Anolis cristatellus*. This finding raises the question of why a recently introduced, demographically bottlenecked population, should present such a weak genetic founder effect. Losses of genetic variation during introduction are often modest (Nei *et al.* 1975) and several reasons may exist for this observation. Multiple introductions, even at low levels, are known to have a strong effect on bottleneck signatures and may be a cause of a weak bottleneck signal (Keller *et al.* 2001). However, this is an unlikely cause of the lack of genetic bottleneck on Dominica. Regular inter-island shipping throughout the Caribbean has been proceeding for hundreds of years with rapidly increasing frequency, and with only a

handful of reported cases of *Anolis* introductions it is unlikely that there have been repeated invasions of a single *Anolis* species on Dominica within seven years. Instead, it is suggested that the intricacies of *Anolis* genetics and life-history characteristics are likely to play a key role in shaping the weak bottleneck signal presented here.

Most genetic variation lost during population bottlenecks is as a result of genetic drift (Nei *et al.* 1975). When the populations remain at small size for few generations, rapid population expansion follows the bottleneck, or the founding population is large, genetic drift is negligible. *A. cristatellus* on Dominica may have retained variation because insufficient generations were spent at small population size to have lost a significant amount genetic diversity. In the *c.* seven years between introduction and genetic characterisation, *A. cristatellus* has undergone a rapid population expansion, accompanied by a similarly rapid range expansion on Dominica. Although generation time is approximately twelve months, females produce eggs every two weeks during the rainy season (Eales, pers.obs) which may last up to eight months. In native ranges, anoles occur at high abundances of up to $0.97/m^2$ (Schoener & Schoener 1980) which are consistent with those observed across much of the *A. cristatellus* Dominican range. As a result, it is likely that the number of generations with a small population size has been few, and consequently, that genetic drift has been minimal for this introduction. Furthermore, although the size of the initial founding population has been cited an important factor contributing to the genetic bottleneck (Veltman *et al.* 1996), it is the amount of genetic variation contained within that population that is most significant. AMOVAs showed that the majority of genetic variation exists within *A. cristatellus* populations, rather than between populations or groups of populations, indicating limited genetic structuring (probably due to high gene flow) even in Puerto Rican populations. Thus, a small founding population sourced from within a native Puerto Rican population will contain a relatively high degree of genetic diversity, facilitating high genetic diversity in Dominican *A. cristatellus*.

One particular aspect of *Anolis* reproductive ecology presents an important mechanism by which genetic diversity can be maintained during a founder event, accounting for the weak bottleneck signal described in this study. The phenomenon of sperm storage has been described in many squamates (Cuellar 1966; Pearse & Avise 2001; Sever & Hamlett 2002) and female *Anolis* have specialised vaginal sperm storage tubes

specifically for this purpose (Fox 1963). Since female *Anolis* ubiquitously mate with multiple males (Tokarz 1998), sperm storage introduces the potential for multiple paternity of offspring. A study by Johnson *et al.* (in review) found that 52% of *A. cristatellus* females that had produced two or more progeny had mated with multiple males, and for *Anolis sagrei* the figure was 81% (Calsbeek *et al.* 2007). The data for the length of time that female *Anolis* are able to store sperm and produce viable offspring ranges from 107 days (*A. sagrei*, Calsbeek *et al.* 2007) to 10 months (*Anolis carolinensis,* Passek 2002). These reproductive characteristics have significant implications for the potential genetic contribution by female *Anolis* that have been sexually active in the source range prior to introduction.

Female *A. cristatellus* can produce up to six viable offspring under isolated laboratory conditions (Eales, pers. obs.), thus each sexually active female can potentially introduce the genotypes of up to six individuals of different paternity to introduced populations. Based on the presence of at least seven sexually mature females in the initially transported founding population (deduced from prior mtDNA analysis, Eales *et al.* 2008b *submitted*), multiple paternity could be responsible for the introduction of up to 42 genotypes in Dominica. These figures are upper estimates, in light of empirical data from *Anolis* females (Calsbeek *et al.* 2007; Johnson *et al.* in review). However, the founding population was not necessarily restricted to seven females, and females with identical mtDNA haplotypes may also have been present in the group transported to Dominica. Together, these factors may result in a much higher initial effective population size for *A. cristatellus* than the actual number transported. The capacity for the introduction of varied paternal genotypes by sexually active females in the initially transported population may be a substantial contributor to the genetic diversity presented in this study. These aspects of squamate reproductive ecology may represent an important mechanism by which introduced populations of this order might minimise the extent of genetic founder effects resulting from demographic bottlenecks.

The genetic signal from a demographic bottleneck is most apparent in the loss of rare alleles (Maruyama & Fuerst 1985), followed by a reduction in the mean number of alleles (Luikart *et al.* 1998). Although there was no significant alteration of the L-shaped allele frequency distribution, fewer alleles (total number of alleles and number of alleles per locus) were present in introduced as compared to native and source

populations, even though sample sizes were larger for Dominican populations. This observation is concordant with lower allelic richness and H_E in Dominican than Puerto Rican populations, and together provide evidence for loss of variation in some genetic diversity measures. However, there was no conclusive and consistent evidence of loss of genetic diversity in introduced populations from pairwise F_{ST} comparisons or group comparisons of H_O, F_{ST}, and F_{IS}.

The use of heterozygote excess to detect a population bottleneck may be limited to severe or very recent bottlenecks (Cornuet & Luikart 1996). Tests for heterozygote excess (relative to the number of alleles in a population) showed no evidence of a founder effect in introduced Dominican populations, and was inconsistent across mutational models, an observation which has also been reported in recent studies (Hufbauer *et al.* 2004; Hawley *et al.* 2006; Aketarawong *et al.* 2007). The Wilcoxon test for the significance of heterozygosity excess was used because it has been cited as the most robust of the significance tests implemented by the program. However, it has most power when 10-25 loci are used (Piry *et al.* 1999), and therefore may be of limited value in this study (five loci). Because microsatellites rarely follow a strict mutational model, programs that rely on mutational models may be of limited value.

Several lines of evidence raise the question as to whether the founding population on Dominica is fully represented by the three sample sites from the putative source area in central Puerto Rico. First, an allele which is present in both Dominican and PR non-source populations was identified. Two explanations may account for this finding. Either the true PR source comprises a larger geographical area than the putative source, or the allele is present in, but was not sampled from the putative source populations. Second, three private alleles were identified on Dominica. High fecundity of anoles (each female produces an egg approximately every 14 days during the rainy season) has led to high abundances on Dominica (numbering into the hundreds of thousands) and given the current estimates of microsatellite mutation rates (in the region of 5×10^{-4}, Goldstein & Schlötterer 1999) it is possible that new alleles may have been created in the *c.* seven years between introduction and genetic characterisation. However, because of the wide distribution of the three private Dominican alleles in populations across the Dominican range, they are likely derived from the PR source, rather than arising from

new mutations occurring in the short time before dispersal of the founder population. Together with results from AMOVAs and Pairwise F_{ST} estimates, these observations provide strong evidence that significant amounts of genetic variation exist within Puerto Rican populations, such that despite substantial sample sizes, not all alleles in a population can be represented by the samples of populations used in this study. A high degree of within-population variation was also inferred by mtDNA analyses: individuals taken from the same population of *A. cristatellus* frequently had different haplotypes, a common observation in other anole phylogenetic studies (Malhotra & Thorpe 2000; Glor *et al.* 2001; Kolbe *et al.* 2004). Consequently, there is no unequivocal evidence to suggest that the three sample sites do not adequately represent the geographic source of the population on Dominica. It is more likely that there is simply a very high degree of within-population variation. Even so, the categorisation of source and native Puerto Rican populations should be treated with caution when interpreting the results.

These results provide valuable insights into the relationship between genetic diversity within source and introduced populations and the success of introductions in the early stages of invasions, information which may be applied to other recent introductions. This study provides a clear representation of the degree of genetic variation present in a recent (less than 10 generation) species introduction at the fine-scale microsatellite level. Evidence presented here suggests that high population genetic diversity present in a species' native range may be transferred to introduced populations with minimal genetic founder effects and result in a highly successful invasion, despite the founding population originating from a limited geographical area. Previous studies have shown that a large amount of genetic variation in introduced *Anolis* populations results from admixture from different geographical areas of the native range (Kolbe *et al.* 2004). Conversely, here it is demonstrated that admixture is not a necessary requirement for observed high genetic diversities in introduced populations. A high degree of gene flow within source areas can help ameliorate founder effects when founding populations originate from a restricted area. It is suggested that sperm storage is a major contributor to the observed genetic diversity in introduced Dominican *A. cristatellus* populations, and this is proposed as an important mechanism in minimising genetic diversity losses during demographic founder events in *Anolis* and other squamates

CHAPTER 4

Revealing the geographic origin of an invasive lizard: the problem of native population genetic diversity

4.1 Abstract

The brown anole, *Anolis sagrei*, is one of the most widespread and successful colonisers of the diverse *Anolis* genus, which comprises *c.* 400 species occurring naturally in Central and South America and the Caribbean. Based on extensive between and within population sampling from a previously published study (334 mitochondrial DNA sequences) and sampling for this study (37 mtDNA sequences), we reconstruct a phylogeny and produce a haplotype network to assign a recently introduced population in St. Vincent, Lesser Antilles to its geographic origin. A single haplotype was present in the St. Vincent population, which was identical to a haplotype from Tampa, FL. This study shows that genetic diversity within native range populations, combined with low frequencies of introduced haplotypes in native ranges, may impair attempts to identify source populations, even despite intensive sampling effort. The absence of mtDNA haplotype diversity suggests a significant genetic founder effect within the St. Vincent population. Low establishment success in St. Vincent *A. sagrei* is most likely the result of stochastic effects on demographic stability, which may have more influence on establishment potential than genetic founder effects in early stage introductions.

4.2 Introduction

As human activity increasingly shapes the distribution and abundance of species at scales ranging from landscape to global, an understanding of the ecological consequences must follow if we are to respond appropriately to conservation issues raised by these biogeographical alterations. The significant and wide-ranging consequences of anthropogenic influences on biogeographic patterns, biodiversity and ecosystem stability are exemplified in human-mediated species introductions. Once established, introduced species can have significant ecological and evolutionary impacts on native species, communities and ecosystems (Elton 1958; Simberloff & Stiling 1996; Mack *et al.* 2000; Sakai *et al,* 2001), and severe consequences for the economic welfare of invaded environments (Pimentel *et al,* 2000). In recent years, it has been increasingly recognised that the genetic diversity and demographic stability of introduced populations are key indicators of the potential for self-sustaining establishment in novel environments (Facon *et al,* 2003; Suarez & Tsutsui 2008). Thus, population genetic and demographic investigations are valuable in indicating the requirement and direction for conservation focus in empirical introductions (Costa-Pierce 2003; Mack *et al,* 2000).

The rapid increase in global anthropogenic movements over the past century has led to an associated dramatic rise in the number of reports of species outside their natural ranges (Flux 1994; Minchin & Moriarty 1998; Greene *et al,* 2002; Norval *et al.* 2002; Clegg *et al.* 2002; Facon *et al,* 2003). Although widespread commercial transportation has provided copious opportunities for species rafting, proportionately few imported populations successfully establish in new environments (Crawley 1986; Shigesada & Kawasaki 1997). Past investigations of this phenomenon have focused on the susceptibility of environments and characterisation of invasive species traits to account for this differential establishment ability (Baker 1965; Ehrlich 1989; Schloesser *et al.* 1996). Furthermore, it has been recently recognised that imported species are frequently exposed to novel selective pressures in the new environment (Lee 2002). Although genetic characterisation of introduced populations (e.g. using mitochondrial DNA sequencing) are based on neutral genetic variation, they can infer whether a degree of genomic genetic diversity exists, in turn indicating a potential for adaptive evolutionary change (Sakai *et al.* 2001). However, stochastic influences on population dynamic processes are also likely to hold significant consequences for establishment potential

(Williamson & Fitter 1996), especially for colonisations that involve a single importation of a small sized propagule.

The identification of the geographic source for introduced non-natives using phylogenetic techniques is fundamental to elucidating the influence of multiple introductions, gene flow and hybridisation on introduction success in addition to the reconstruction of introduction histories (Williamson 1996; Tsutsui *et al.* 2000; Neuffer & Hurka 1999; Durka *et al.* 2005; Lavergne & Molofsky 2007). Moreover, genetic analyses of introduced founder populations have valuable applications outside the field of biological invasions. Molecular investigations of founder populations also provide an invaluable model for the genetic consequences of demographic bottlenecks faced by many endangered species and fragmented populations (Frankham *et al.* 2002), and contribute to the understanding of natural colonisation and radiation processes.

Human-mediated invasions have often been documented within decades of initial import (Facon *et al.* 2003; Williams 1969). It is less common, however, to discover an invasion less than five years from the initial importation. Reports of the non-native *Anolis sagrei* (Sauria: Iguanidae) on the island of St. Vincent, Lesser Antilles, dating back to around 2003, have provided a fortuitous opportunity for investigating the genetic processes involved in the early stages of introductions. Anoles have been widely transported throughout the Caribbean both by natural (Calsbeek & Smith 2003; Stenson *et al.* 2004) and human mediated dispersal (Campbell 1996). However, the St. Vincent discovery is rare in that the single point of import is known, its current range within the island is small, and two native competitors (*A. trinitatis* and *A. griseus*) already occupy and partition *Anolis* niche space on the island (Schoener & Gorman 1968).

Of all the Caribbean anoles, the brown anole (*A. sagrei*) is perhaps the most widespread and successful at colonisation (Williams 1969). A native of the Bahamas, Cuba and the Cayman islands, introduced populations have been reported in Florida (Bell 1953), Jamaica (Williams 1969), Grand Cayman (Minton & Minton 1984), Texas (Conant & Collins 1991), Mexico (Lee 1992), Louisiana (Steven & Lance 1994), Georgia (Reznick & Ghalambor 2001), Hawaii (McKeown 1996), Belize (Rodriguez Schettino 1999), and most recently, Grenada (Greene *et al.* 2002) and Taiwan (Norval *et al.* 2002). An extensive phylogeographical study by Kolbe *et al.* (2004) identified the source of many

of these populations using mitochondrial DNA data, and indicated that the high genetic diversity in introduced populations may result from multiple introductions from genetically different native populations. Invasive populations of *A. sagrei* can reach high population densities, expand their range rapidly (Losos *et al.* 1993) and have been shown to be competitively superior to native congenerics (Campbell 1996).

In this study, molecular sequencing techniques are used to reveal the source population for introduced *A. sagrei* in St. Vincent. A phylogeny of *A. sagrei* was produced from mitochondrial DNA (mtDNA) sequence data comprised of haplotypes obtained from new sampling in St. Vincent and published sequences for this species from throughout the native and introduced range (Kolbe *et al.* 2004). Haplotype networks and phylogeographic analysis reveal the likely geographical origin for the introduced population. The impact of native range within-population genetic diversity on the reliability of identifying geographical source populations is discussed. The genetic diversity of the St. Vincent population is compared with that of other (including *A. sagrei*) introductions, and the relative importance of genetic diversity and stochastic influences for establishment ability is discussed.

4.3 Methods

4.3.1 Sample collection

Thirty-six tail tip biopsies, preserved in 95% ethanol, were taken from hand-caught and released lizards at the site of invasion, adjacent to the dock in the industrial port of Campden Park, on the South-West coast of St. Vincent, Lesser Antilles, Fig. 1.6; Appendix I. The range was recorded using a handheld Garmin 60CS GPS. Samples were collected throughout the limited range of the introduced population (approximately $400m^2$). To locate the geographic origin of the introduced population, *A. sagrei* mtDNA sequences representing areas from across the native (74 populations) and introduced (29 populations) range were used for comparison with St. Vincent samples, Fig. 4.1 . These mtDNA sequences were deposited in GenBank by Kolbe *et al.* (2004) accession numbers AY655172-484; DQ846752-771, Appendix III. Eight outgroup haplotypes were used comprising three congeneric Cuban natives; *A. homolechis, A.bremeri* and *A.quadriocellifer* (GenBank accession numbers AY655164-171).

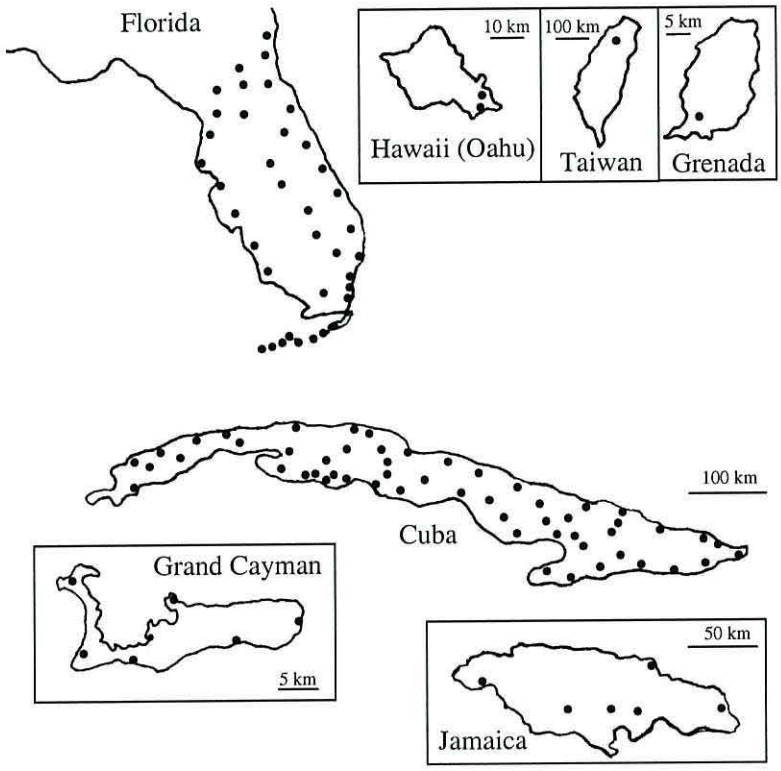

Figure 4.1

Map showing localities sampled by Kolbe *et al.* (2004), altered from Kolbe *et al.* (2004). Areas sampled for which locality information is unavailable are Louisiana, Texas and Belize.

Figure 4.2. (following page)

(a) Map showing geographic distribution of *A. sagrei* haplotypes within the SE Cuba, WC Florida, W Jamaica, St. Vincent clade. Grey squares indicate haplotypes from Cuba, open squares indicate haplotypes from Florida/St. Vincent, lined squares indicate haplotypes from Jamaica. 257 *A. sagrei* haplotypes from 74 native range populations and 77 haplotypes from 29 introduced populations from Kolbe *et al.* (2004) were included in this study (not marked on the map).

(b) Network analysis showing genealogical relationship between *A. sagrei* haplotypes, shown in (a), the number of nucleotide substitutions between haplotypes is shown by the number of dots connecting them in the network. (Haplotype Identifiers: StV/TII= St. Vincent /Tampa, Florida II; TI= Tampa, Florida I; StPI=St. Petersburg, Florida I; StPII=St. Petersburg, Florida II; JMI = Manchester, Jamaica I; JMII = Manchester, Jamaica II; JWI = Westmoreland, Jamaica I; JWII=Westmoreland, Jamaica II; PI =Portillo, Cuba I; P II= Portillo, Cuba II; NML I= N Media Luna, Cuba I; NML II= N Media Luna, Cuba II; NML III= N Media Luna, Cuba III).

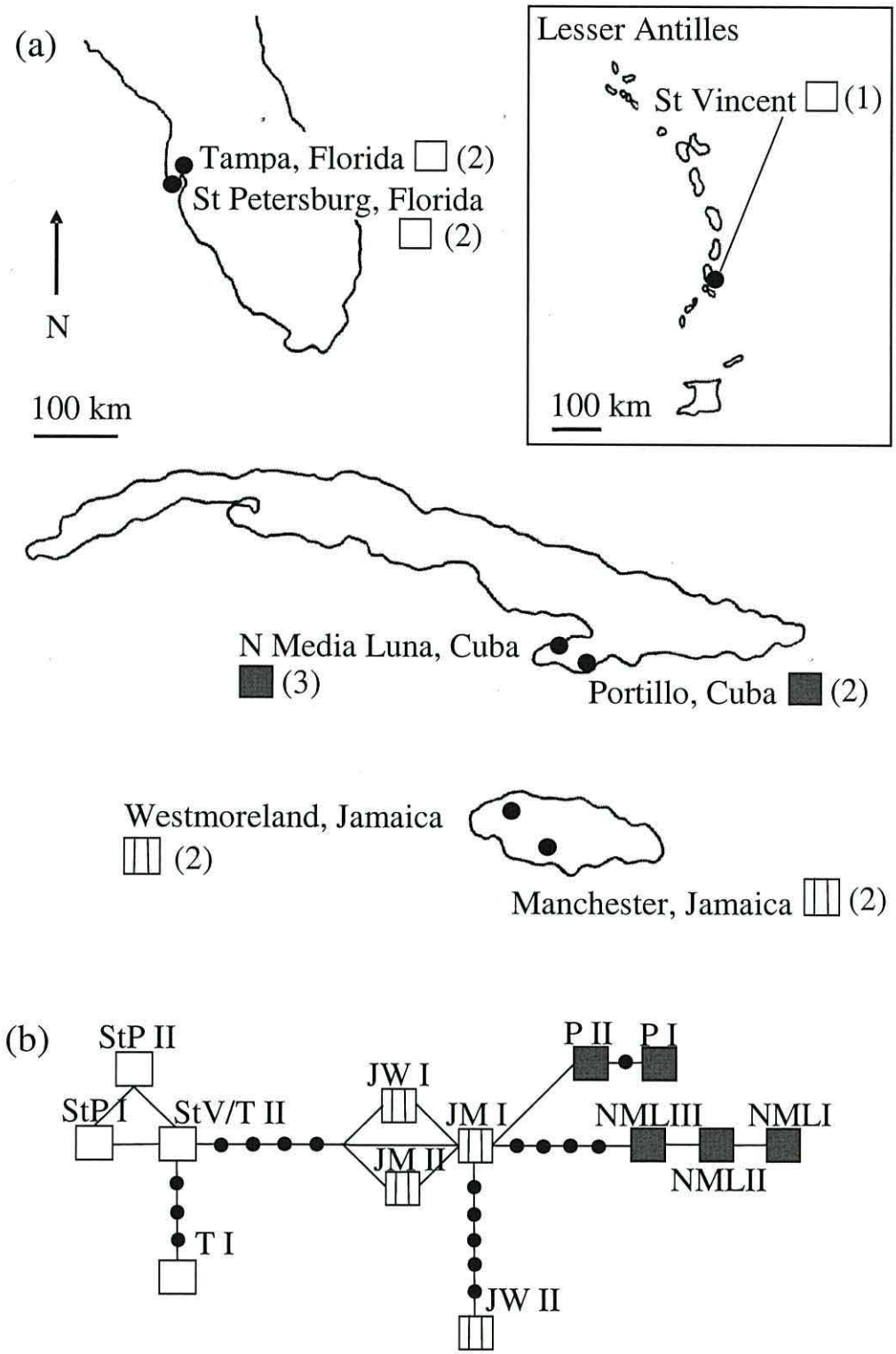
(a)
Lesser Antilles
St Vincent (1)
Tampa, Florida (2)
St Petersburg, Florida (2)
N
100 km
100 km
N Media Luna, Cuba (3)
Portillo, Cuba (2)
Westmoreland, Jamaica (2)
Manchester, Jamaica (2)
(b)
StP II
StP I
StV/T II
JW I
JM II
JM I
P II
P I
NMLIII
NMLI
NMLII
T I
JW II

Figure 4.2.

4.3.2 DNA extraction and amplification

Total genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN) following the manufacturer's protocol for animal tissues. Approximately 1200bp of mtDNA, including NADH 2 and two flanking tRNAs ($tRNA^{Trp}$ $tRNA^{Ala}$) was amplified using the primers H5730 (Glor *et al.* 2004) and L4437b (Macey *et al.* 1997). The amplifications were performed in a total volume of 25 µL using 50 ng total genomic DNA, 20µl (1.5mM $MgCl_2$) ReddyMix® PCR Master Mix (ABGene Surrey, UK) and 1µM of each primer. PCR amplifications were performed using an MJ Research PTC-200 thermal cycler (Waltham, MA) using the following temperature cycling profile: a 15-min denaturation step at 95°C followed by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 50°C and extension for 1 min at 70°C. A final step at 72°C for 10 min followed the 30 cycles. Unincorportated deoxynucleotides and primers were removed using Promega 10xSAP and New England BioLabs Exonuclease I, following the manufacturer's protocols. The forward and reverse primers were used for sequencing using dye-labelled terminators (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit) and subsequently run on an ABI Prism 377 DNA sequencer.

4.3.3 Preliminary data analysis

Sequences were aligned using CODONCODE ALIGNER 1.2 (CodonCode Co.) and replicated haplotypes were excluded from the analysis in order to reduce computational time. The substitutional model that best fit the data was assigned according to distance analyses performed using the Akaike Information Criterion function of MODELTEST 3.0 (Posada & Crandall, 1998), and the chosen model was then used for subsequent analyses. McDonald and Kreitman's (1991) test was used to evaluate the possibility of non-neutral evolution of the ND2 gene (Ballard & Kreitman 1995). The genetic data analysis software packages PAUP*4.0b10 (Swofford 1998) and MRBAYES version 3.1 (Huelsenbeck & Ronquist 2001) were used to reconstruct phylogenetic trees by maximum parsimony (MP) and Bayesian methods respectively. Analyses included 334 unique haplotypes (excluding 8 outgroups) from native and introduced populations across the native and invasive ranges, between one and ten unique haploypes per

population. This strategy enabled all available information to contribute towards producing the most structurally informative phylogeny possible.

4.3.4 Phylogenetic and network analysis

In all analyses, all sites were equally weighted. MP was performed using a random addition heuristic search with tree–bisection–reconnection (TBR) with 1000 random addition sequence replicates. Bootstrapping (1000 replicates) was performed to obtain a relative measure of node support for the resulting MP tree (Felenstein 1985). The model of evolution used for Bayesian analysis was estimated by MODELTEST 3.0 and the parameter values were estimated by MRBAYES. Four chains were run, with 2 million generations, sampling the chains every hundred generations. Time to stationarity was estimated by plotting tree log-likelihood score against generation number and determining the number of generations until the values reached an asymptote. The initial 25% of trees that were generated prior to stationarity were discarded as the "burn-in". A majority rule consensus tree ('Bayesian' tree) was calculated from the posterior distribution of trees, and the posterior probabilities calculated as the percentage of samples recovering any particular clade (Huelsenbeck & Ronquist 2001). Three further independent Bayesian analyses were run to check for local optima.

A haplotype network was constructed using the network building software TCS (Clement *et al.* 2000) which uses a statistical parsimony procedure (Templeton *et al.* 1992). TCS produces a network linking different haplotypes only if they have a 95% probability of being justified by the parsimony criterion. Although network analysis is traditionally used to show genealogical relationships between haplotypes within populations (Templeton *et al.* 1992), it gives a useful visual indication of the number of steps (nucleotide differences) between haplotypes. Within-population nucleotide diversity (π) was calculated using MEGA3.1 (Kumar *et al.* 2004) for all populations sampled with more than one unique haplotype.

4.4 Results

4.4.1 Preliminary analysis

A 1194bp fragment of mtDNA from a total of 342 sequences were used in the analysis. A single haplotype was identified from 36 sequenced St. Vincent *A. sagrei* individuals. This haplotype was identical to a haplotype from Tampa, Florida, and the duplicate sequence was removed from further analyses. No insertions, deletions or stop codons were found in the data and McDonald and Kreitman's (1991) test showed no significant deviation from neutrality. Of the 1194 sites, 666 were variable, 547 of which were parsimony-informative.

4.4.2 Phylogenetic analyses and haplotype network

Bootstrapped MP and Bayesian analyses gave congruent tree topologies with similar, high bootstrap values, summarised in the Bayesian tree presented in Fig. 4.3. Both trees group St. Vincent *A. sagrei* in a clade with 12 haplotypes from six localities across South East Cuba, West Central Florida and West Jamaica (shaded clade shown in Fig. 4.3; inset). Phylogenetic tree support was high for the clade (posterior probability 0.98, MP bootstrap support 81%, Fig. 4.3). Support for the 4 taxa clade grouping the St. Vincent haplotype with Tampa and St. Petersburg was high (posterior probability 1.00, MP bootstrap support 85%). The geographical distribution of *A. sagrei* haplotypes within the SE Cuba, WC Florida, W Jamaica clade is presented with geneological relationships among these haplotypes obtained from the network analysis in Fig. 4.2(b). This clade was unconnected to other haplotypes because only connections with a probability higher than 95% are shown. Network analysis separates St. Vincent/Tampa II from St. Petersburg by 1 step, Tampa I by 4 steps, W Jamaica by 5 steps and from SE Cuba by 8 steps, Fig. 4.2(b). Fig. 4.4 shows the within-population nucleotide diversity. for all populations included in this study Native populations have typically higher within-population nucleotide diversities, whilst introduced populations have typically lower diversities (Fig. 4.4). A notable exception is Florida, which contains among the oldest established of the introduced populations and has been colonised by multiple introductions from across the native range (Kolbe *et al.* 2004).

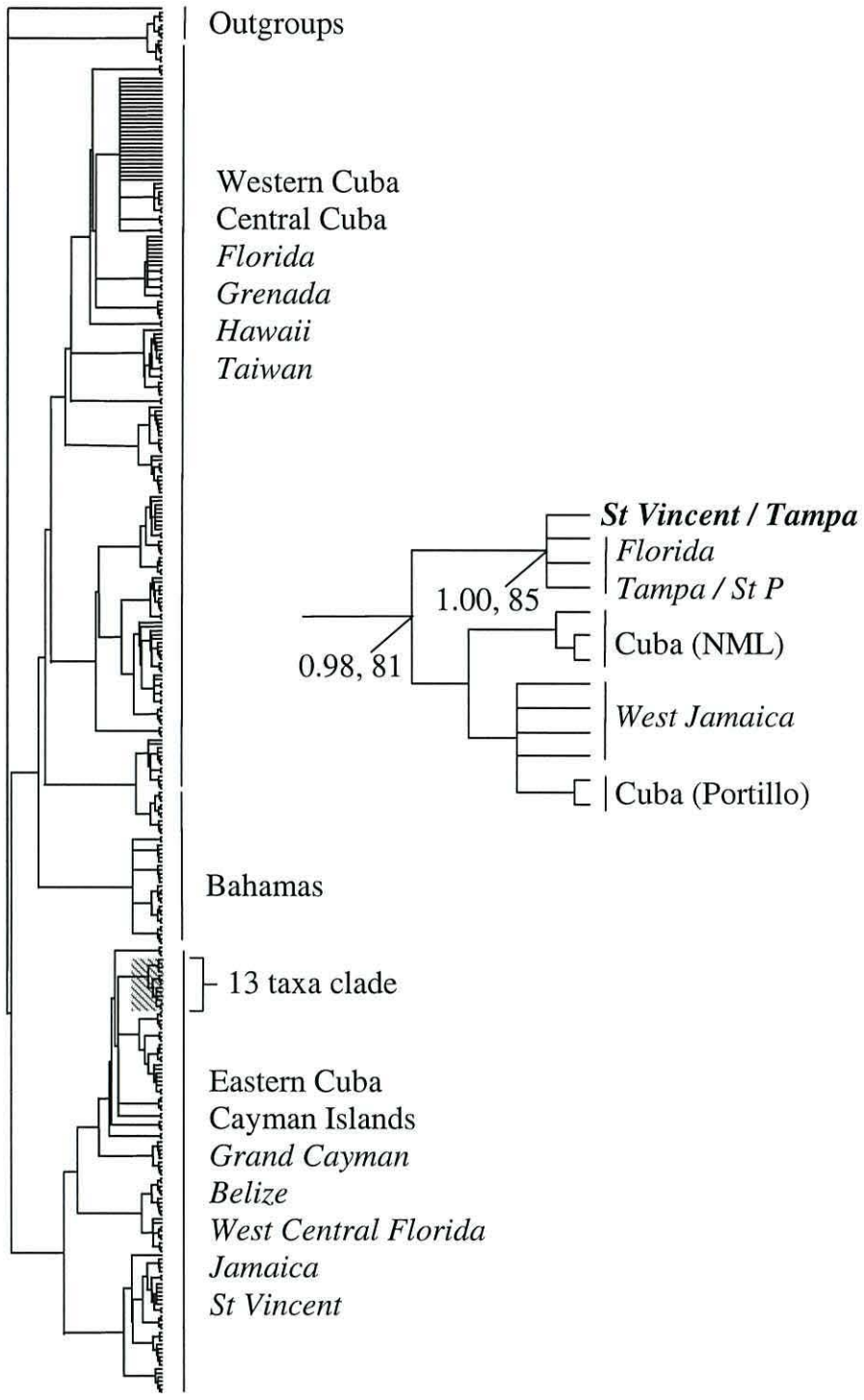

Figure 4.3.

Bayesian phylogram of the ND2 region sequence data for *A. sagrei*, N=342 haplotypes. Shaded clade is expanded in inset. Support for nodes are presented in the order Bayesian posterior probability value and maximum-parsimony bootstrap percentage. MtDNA haplotype data from Kolbe *et al.* (2004).

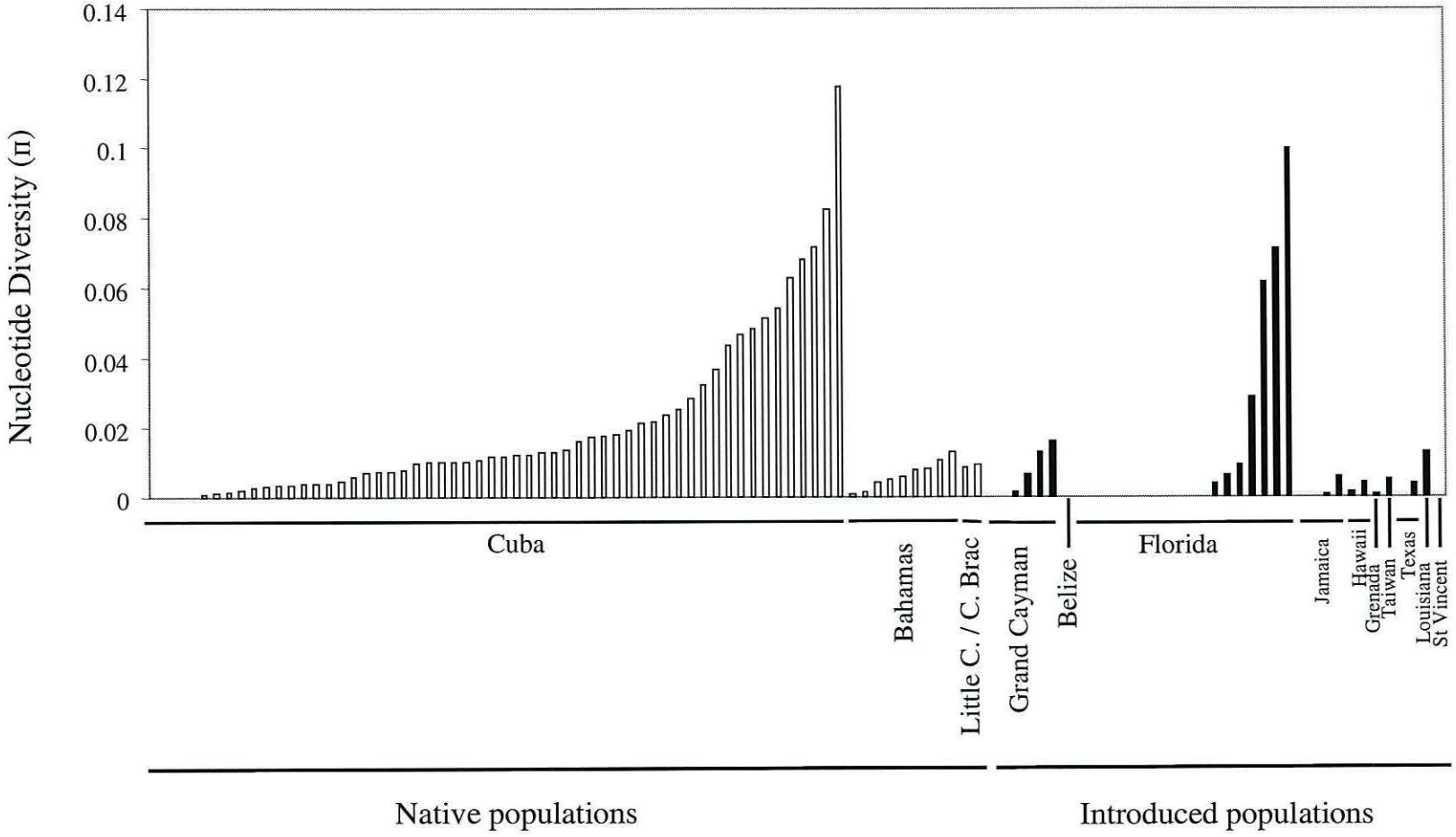

Figure 4.4.
Histogram of within-population nucleotide divergence (Π) for 1194bp within the mtDNA ND2 region for all native and invasive *A. sagrei* populations included in the study, mtDNA haplotype data from Kolbe *et al.* (2004). Populations are grouped by country / state. Populations with no bar contain a single haplotype (4 populations from Cuba; 2 from Grand Cayman; Belize; 11 from Florida; 1 from Texas; 2 from Jamaica and St. Vincent).

Table 4.1.

Within-population nucleotide diversity (π) for each native and introduced range population of *Anolis sagrei* in this study that were found to contain more than a single haplotype. A single haplotype was present in the following 22 localities, not included in the table: Playa Giron, Jaguani, El Yunque, Playa Larga (Cuba); Frank Sound, George Town (Grand Cayman); Belize; Ocala, Port Richey, Naples, Plantation Key, Okeechobee, Stock Island, Red Road, Pahokee, Miami, Gainsville, Cape Canaveral (Florida); St Catherine, Portland (Jamaica); Corpus Christi (Texas); St. Vincent.

Nucleotide diversity (π)	Population	Location	Number of haplotypes
0.00085	Gibara	Cuba	2
0.00113	W. Bayamo	Cuba	3
0.00169	N. Moron	Cuba	3
0.00186	Holguin	Cuba	4
0.00282	Santa Lucia	Cuba	3
0.00322	Las Tunas	Cuba	5
0.00338	Limoncito	Cuba	3
0.00366	Moa	Cuba	4
0.0039	Santo Tomas	Cuba	4
0.0039	Cardenas	Cuba	5
0.00395	Santi Spiritus	Cuba	3
0.00479	S. Manicargua	Cuba	4
0.00593	Santiago de Cuba	Cuba	2
0.00719	Caibarien	Cuba	4
0.00732	Juragua	Cuba	3
0.00761	San Blas	Cuba	3
0.00803	Sagua la Grande	Cuba	4
0.00981	Santo Domingo	Cuba	5
0.01016	Topos de Collantes	Cuba	2
0.01016	Guantanamo	Cuba	3
0.01043	N. Aguada	Cuba	3
0.01048	Corralillo	Cuba	5

0.01057	Cascorro	Cuba	4
0.01171	San Jose de las Lajas	Cuba	4
0.01185	N. Media Luna	Cuba	5
0.01211	Mulata	Cuba	4
0.01242	W. Florida	Cuba	3
0.01296	S. Esmerelda	Cuba	3
0.01321	Cauto	Cuba	5
0.01368	Portillo	Cuba	4
0.01609	Mariel	Cuba	2
0.01724	Rio Cauto	Cuba	5
0.0178	La Habana	Cuba	2
0.01837	W. Trinidad	Cuba	5
0.01919	Jicarita	Cuba	5
0.02145	W. Mayari	Cuba	3
0.02164	Soroa	Cuba	5
0.02371	Jobabo	Cuba	3
0.02536	W. Sigua	Cuba	4
0.02832	E. Yaguajay	Cuba	5
0.03226	S. Camaguay	Cuba	5
0.03692	Santa Clara	Cuba	5
0.04339	Caracusey	Cuba	3
0.04657	Maisi	Cuba	2
0.04837	Ciego de Avila	Cuba	6
0.05149	Vertientes	Cuba	5
0.0541	Puerto Padre	Cuba	3
0.06279	Minas	Cuba	4
0.06785	El Cenote	Cuba	5
0.07143	San Juan y Martinez	Cuba	3
0.08222	Perico	Cuba	4
0.1175	Cortes	Cuba	2
0.00084	Crooked Island	Bahamas	2
0.00169	San Salvador	Bahamas	4
0.0045	South Bimini	Bahamas	3

0.00521	South Andros	Bahamas	4
0.00591	Great Abaco	Bahamas	5
0.00788	Berry Islands	Bahamas	4
0.00828	Staniel Cay	Bahamas	5
0.01082	Exuma	Bahamas	5
0.01301	Grand Bahama	Bahamas	5
0.00874	Little Cayman	Cayman Islands	9
0.00961	Cayman Brac	Cayman Islands	10
0.00169	Sunnyfield	Grand Cayman	2
0.00676	South Coast	Grand Cayman	3
0.01296	Cayman Kai	Grand Cayman	3
0.01606	Turtle Farm	Grand Cayman	3
0.0039	Lower Matecumbe Key	Florida	2
0.00615	Coral Gables	Florida	3
0.0093	Fort Myers	Florida	2
0.02874	Big Pine Key	Florida	3
0.06165	St Petersburg	Florida	6
0.07114	Tampa	Florida	9
0.09983	Lake Worth	Florida	2
0.00085	Manchester	Jamaica	2
0.00592	Westmoreland	Jamaica	2
0.00169	Lanikai	Hawaii	4
0.00423	Hawaii Kai	Hawaii	2
0.00085	Grenada	Grenada	2
0.00507	Taiwan	Taiwan	2
0.0041	Houston	Texas	2
0.01308	New Orleans	Louisiana	7

4.5 Discussion

Identical mtDNA haplotypes from St. Vincent and Tampa II, FL supports a Tampa origin for the St. Vincent population. Additionally, both MP and Bayesian tree topologies converge and group St. Vincent into a well-supported clade with 3 haplotypes from Tampa and St. Petersburg, FL, localities within small geographic distances (20 km). Network analyses group St Vincent, St. Petersburg and Tampa together, separated from the next closest Jamaican haplotype by 5 steps, further supporting Tampa, FL as the source area for the St. Vincent population. Commercial container imports have been routinely received at the Campden Park dock, St. Vincent from the Northern Florida area during the period when the introduction occurred (F. Providence, pers. comm.) whilst there is no evidence for regular imports from any other of the native or introduced *A. sagrei* ranges, providing additional evidence for a Florida origin for introduced St. Vincent *A. sagrei*.

Although identified in Florida, it is surprising that the St. Vincent/Tampa II haplotype was not also found in the native range, despite the use of data from a comprehensive study of intensive sampling effort. The native range sampling comprised 257 haplotypes from 74 populations (from Kolbe *et al.* 2004), none of which matched the St. Vincent/Tampa II haplotype. In accordance with reports that introduced populations of *A. sagrei* have been in Florida since the late 1800's (Williams 1969), it is improbable that the haplotype would have been lost in the native range though drift within such a timescale, and similarly unlikely that the same haplotype has been formed in the invasive range through mutation during this time. Therefore, the most probable explanation for the situation is that the haplotype exists in the native range but is absent from the sample set. This absence may be explained by the presence of multiple haplotypes and high nucleotide diversities within native range populations (Fig. 4.4; Table 4.1). Even thorough sampling will detect only a subset of the most frequently occurring haplotypes in these populations, with those at lower frequencies being overlooked. Following this logic, it is plausible, though perhaps less probable, that the St. Vincent population may originate from a source population in the native range which contains the St. Vincent/Tampa II haplotype at a low frequency.

A recent study has also recognised that native population genetic diversity and sampling efficiency can substantially affect the accuracy of putative source population identification (Muirhead *et al.* 2008). In a simulation using published data sets, both the degree of genetic differentiation between source populations, and the number of source populations included in the study were found to significantly impact the accuracy of source population assignment. Furthermore, small sample sizes affected population assignment accuracy most when introduced populations comprised of dominant or fixed haplotypes that were at low frequencies in source populations, (Muirhead *et al.* 2008). For this study, the sample size in the introduced St. Vincent range was high (36 individuals), and a large number of potential source populations were sampled (74 native populations and 29 populations across the introduced range). However, in accordance with Muirhead *et al.* (2008), because the haplotype was fixed in the introduced range, and genetic differentiation between potential source populations was high (Fig. 4.4; Table 4.1), the estimation of the putative source population should be treated with some prudence.

Assuming a Tampa origin for *A. sagrei* in St. Vincent, this recent importation represents a secondary introduction event, i.e. originating from an introduced rather than a native population. Other recent secondary introductions originating from Florida *A. sagrei* have been shown to retain a proportion of the genetic variation found within Floridian populations (up to 7.8 % within-population nucleotide divergence, Kolbe *et al.* 2004). In contrast we demonstrate that despite high within-population variation in the source population, Tampa (7.1% within-population nucleotide divergence, Table 4.1), the introduced population of *A. sagrei* in St. Vincent, which contains a single mtDNA haplotype has experienced the most severe genetic depauparation of any of the documented global *A. sagrei* introductions. Such an absence of genetic diversity has rarely been documented in other introduced populations, (although see Puillandre *et al.* 2008; Kliber & Eckert 2005; Poulin *et al.* 2005; Amsellem *et al.* 2000; Cristescu *et al.* 2001), although variation in more sensitive population genetic markers may represent severe founder effects (e.g. microstatellites, Hawley *et al.* 2006). For *A. sagrei* in St. Vincent, the implied genetic founder effect is likely to be a result of colonisation history processes, directly via a demographic bottleneck, and augmented by the early stage of the St. Vincent introduction, which was sampled before any subsequent, additional introductions may have had an opportunity to occur.

Recent investigations have demonstrated that newly founded populations containing high levels of genetic diversity have shown rapid evolutionary responses to local conditions (Costa-Pierce 2003; Maron *et al.* 2004; Ayllon *et al.* 2006). In these empirical examples, diversity often arises from the admixture of haplotypes originating from multiple introductions. In recently introduced populations that have undergone founder effects with no admixture, there may be limited potential for adaptive responses (Barrett & Kohn 1991; Suarez & Tsutsui 2008, although see Rasner *et al.* 2004; Tsutsui *et al.* 2000). Whilst some additive genetic variance may still be available to selection following demographic bottlenecks (for example, via epistasis, Carson, 1990), in populations with severe founder effects, the minimal amounts of available variance present are unlikely to enable adaptive genetic responses (Frankham *et al.* 2002; Allendorf & Lunquist 2003).

However, influences other than genetic diversity are probably more significant in the establishment of self-sustaining populations during the early stages of introduction. The initial founding population size for *A. sagrei* in St. Vincent is likely to be small because a single haplotype was introduced from a source population that contained high within-population genetic diversity (7.1% mean pairwise mtDNA sequence divergence). Typically small-sized founding populations are subject to stochastic influences on the basic processes of population dynamics. Chance events (for example, the emergence of disease or sudden fluctuations in numbers of predators and competitors) may result in reduced fecundity or an increase in the mortality rate of introduced populations (Williamson & Fitter 1996). The subsequent reduction in population size, if sustained and accompanied by other factors detrimental to population success, can lead to local extinction. Between 2005-2007, population densities of *A. sagrei* at Campden Park dock, St. Vincent had diminished substantially in accordance with a population crash (Eales, pers. obs.). Because of the short time (*c.* 5 years/generations) since importation, it is likely that stochastic effects on population dynamics are responsible for the population crash, rather than a limited ability to adapt to the novel environment due to low genetic diversity.

This study identifies a single mtDNA haplotype in a recent anthropogenically imported population of *A. sagrei* on St. Vincent, identical to a haplotype from Tampa, FL,

suggesting that Tampa is the geographical source for the introduction. However, it is also shown that multiple haplotypes with high nucleotide diversities within native range populations, combined with low frequencies (rareness) of introduced haplotypes in native ranges can impair attempts to identify source populations, even despite thorough sampling within the introduced range (36 individuals over approximately 400m^2). Thus, both phylogenetic structure and within-population genetic diversities must be appreciated to identify the likely geographical source for introduced populations. The findings of this study are consistent with Muirhead *et al.* (2008) in recommending careful consideration of sampling design to avoid inaccurate assignment to putative source populations due to sampling errors. The data presented here demonstrate, in contrast to previous genetic characterisation of introduced *A. sagrei* (Kolbe *et al.*, 2004), and *A. cristatellus* populations (Eales *et al.* 2008a), that founder populations can exhibit extremely low haplotype diversity despite high source population genetic diversity, probably as a result of colonisation history. Finally, this study suggests that although the genetic characterisation of introduced populations can suggest the potential for adaptive responses to novel environments, stochastic processes may be more likely to affect establishment success in early-stage introductions by affecting population dynamic processes.

CHAPTER 5

General Discussion

This thesis investigates how the interacting roles of historic, genetic and evolutionary processes affect the establishment success of colonisations, using two early-stage *Anolis* introductions as models. Since each of the three data chapters explore the findings separately, this general discussion will take a holistic approach. The findings of all three chapters are related to colonisation theory and previous studies of natural, anthropogenic and experimental species introductions. The wider relevance of this thesis for invasive species management, particularly for predicting the establishment ability of recent colonisations, is discussed.

5.1 Interactive roles of historic, genetic and evolutionary processes during colonisation

5.1.1 Colonisation history: propagule pressure and colonisation pathways

Colonisation history encompasses propagule pressure (defined as the number of individuals introduced and the frequency of introduction events, Williamson 1996; Kolar & Lodge 2001), the geographical source of propagules, and the timing of introduction events. Predictions from theoretical population biology (MacArthur & Wilson 1969; Richter-Dyn & Goel 1972) and empirical evidence (Forsyth & Duncan 2001; Colautti *et al.* 2006), suggest that propagule pressure is positively associated with establishment success. In the majority of empirical studies in natural systems, multiple introduction events have either been documented (Duggan *et al.* 2006; Ficetola *et al.* 2008; Mikheyev *et al.* 2008), or cannot be discounted due to inaccurate, unavailable or unreliable historical evidence (Jeschke & Strayer 2006; Thulin *et al.* 2006). Since both mtDNA and anecdotal evidence of colonisation history suggest a low likelihood that subsequent introduction events occurred for either of the *Anolis* colonisations studied in this thesis, propagule pressure here is represented by propagule size alone.

Although other studies have provided experimental evidence (Ebenhard 1989; Berggren 2001; Ahlroth *et al.* 2002), this thesis provides evidence from an early stage colonisation of a natural system to suggest a positive association between propagule size and establishment success. However, it is recognised that the association is not rigorously testable because there are no replications of either species introduction. Unlike experimental introduction studies which can set up replicate experiments, manipulate propagule size and control for variation in environmental factors, evidence from this thesis is based on information from anthropogenically-mediated colonisations in natural systems. Inferences on viable propagule sizes for successful establishment by *Anolis* can be made from previous studies that experimentally introduced single propagules of *A. sagrei* onto small Caribbean islands. The experiments were designed to investigate response to habitat variability (Losos & Spiller 1999), biotic interactions (Losos *et al.* 1997), and the effect of island size on establishment ability (Schoener & Schoener 1983). Establishment success was high for experimentally introduced propagules that consisted of two male and three gravid females on each island (Losos & Spiller 1999). In an experimental study across 14 islands initiated by Schoener & Schoener (1983) most of the introduced propagules (all of which had a 2:3 male: female ratio) persisted for a minimum of 10 years (Losos *et al.* 1997), and propagule size (either five or ten individuals) did not have an effect on establishment potential (Schoener & Schoener 1983). Propagules introduced onto very small islands (less than 5 $\times 10^2$ m^2) failed to establish (Schoener & Schoener 1983), although this finding holds minimal relevance for this study, because the island sizes of Dominica and St. Vincent are orders of magnitude larger than of the islands used in the experiment.

These studies indicate that *Anolis* propagules with an minimum of three female founders can have moderate or high establishment potential. Because of their high fecundity (each female produces an egg approximately every 14 days during the long rainy season), the majority of sexually mature *Anolis* females are likely to be gravid at the point of introduction. This implies that the total number of individuals provided by an introduced propagule (and their genetic contribution to the introduced population) may be greater than the number of adult individuals in the transported propagule. For introduced *A. cristatellus* in Dominica, establishment success is high (rapid population and range expansion) and the estimated propagule size is moderate (microsatellite evidence indicates a minimum effective founder population size in the region of 19-25

individuals, although a higher actual number is probable, and mtDNA evidence suggests a minimum of seven females in the founder population). In contrast, establishment of *A. sagrei* in St. Vincent is failing (diminishing population and range extent estimates) and the estimated propagule size is very low (minimum of one female founder from mtDNA evidence). These associations suggest that the propagule size, independent of propagule number, may be a significant factor in predicting establishment success. The basis of the associations may result from a direct demographic advantage and/or the beneficial genetic correlates of larger founding population size.

Short-term stochastic fluctuations in population size and density are a feature of many small, isolated populations and can ultimately result in population extinction (Sax & Brown 2000; Duncan *et al.* 2003). Small populations, for example, *A. sagrei* in St. Vincent, are particularly susceptible to establishment failure resulting from stochastic influences, such as environmental instability. Furthermore, founding populations are often faced with novel environments that can impose new selection pressures (Maron *et al.* 2004). The strength and direction of these selective pressures may result in selective mortality, increasing the probability of population extinction. The genetic attributes of larger propagules are likely to play a significant role in facilitating establishment under such conditions. According to theory (Mayr 1963), larger propagules are likely to contain higher genetic diversity (e.g. Baker & Mooed 1987) and are less susceptible to the detrimental effects of inbreeding and founder effects than small propagules. A direct comparison of the population genetic level variability between the two introduced *Anolis* was not undertaken using microsatellite markers, because the microsatellite primers developed for *A. cristatellus* did not cross-amplify in *A. sagrei* individuals. However, the number of mtDNA haplotypes present in each introduced population provided an indication of genetic diversity, and some support for the hypothesis that introductions involving small sized propagules (*A. sagrei* in St. Vincent) are likely to contain less genetic diversity than those which originate from larger propagules (moderately sized propagule of *A. cristatellus* in Dominica). Sufficient genetic diversity potentially enables adaptive responses to the novel selective forces associated with establishment in new environments (Reznick *et al.* 1997).

The work presented in this thesis suggests that during the early stages of colonisation, propagule size may be a predictor of establishment potential, probably because of the

importance of overcoming demographic stochasticity and the negative genetic correlates of small propagule size. Both *Anolis* colonisations were sampled at an early stage, and it is most unlikely that there has been an opportunity for subsequent introduction events in either case. Multiple introduction events may be more important in overcoming the influence of spatially structured ecological forces (e.g. climatic and biotic interactions) across introduced ranges, thus are probably more influential for invasion success in latter stages of introduction following range expansion after early establishment. The dramatic difference in establishment success between the two studied colonisations suggests that the shared life-history traits and ecological characteristics shared by both *Anolis* colonisations hold less importance for establishment potential if propagules are small. Although the typically high fecundity of *Anolis* probably enabled rapid colonisation by *A. cristatellus*, the same high fecundity did not enable establishment by *A. sagrei*. The contrast in establishment success implies that during early colonisation, even relatively subtle differences in propagule size may predict establishment success or failure.

This thesis also demonstrates the usefulness of mitochondrial DNA (mtDNA) analyses in the reconstruction of colonisation pathways (in addition to determining the size of founding propagules), in the absence of detailed information about the geographic origin of introduced populations. Phylogenetic reconstruction based on mtDNA data revealed that *A. cristatellus* in Dominica originated from a resticted area of the native Puerto Rican range. The presence of a single mtDNA haplotype of *A. sagrei* in St. Vincent identical to a haplotype from Tampa, Florida suggested that the St. Vincent population originated from a single, secondary introduction (Florida *A. sagrei* was originally introduced from Cuba, from mtDNA phylogenetic reconstruction presented in Kolbe *et al.* (2004)). However, the results of phylogenetic analyses to indicate geographical source populations may not always be accurate if high genetic diversity (i.e. multiple haplotypes) exists within native range populations. Even if sampling effort is high, haplotypes that are present at low frequencies within native range populations may be overlooked. This can lead to erroneous conclusions when considering the geographical source of introduced populations. This finding recommends prudence in the interpretation of mtDNA analyses for identifying source populations.

In accordance with reports that introduced populations of *A. sagrei* have been in Florida since the late 1800's (Williams 1969), it is improbable that the haplotype would have been lost in the native range though drift within such a timescale, and similarly unlikely that the same haplotype has been formed in the invasive range through mutation during this time. Therefore, the most probable explanation for the situation is that the haplotype exists in the native range but is absent from the sample set. This absence may be explained by the presence of high nucleotide diversities (multiple haplotypes) within native range populations, (Fig. 4.3; Table 4.1). Even thorough sampling will detect only a subset of the most frequently occurring haplotypes in these populations, with those at lower frequencies being overlooked. Following this logic, it is plausible, though perhaps less probable, that the St. Vincent population may originate from a source population in the native range which contains the St. Vincent/Tampa II haplotype at a low frequency.

The problem of native population genetic diversity and sampling efficiency having a significant effect on the identification of putative source population has recently been recognised (Muirhead *et al.* 2008). Using simulations based on published data sets Muirhead *et al.* (2008) found that the degree of genetic differentiation between source populations (and the number of source populations included in the study) had significant impacts on the accuracy of source population assignment. The errors associated with small sample sizes were most acute when introduced populations comprised of dominant or fixed haplotypes that are rare in source populations. Thus, it is apparent that sampling design must be considered to avoid incorrect assignment to putative source populations due to sampling errors. This study has attempted to minimise sampling errors by using large introduced population sample sizes (36 *A. sagrei* individuals and 98 *A. cristatellus* individuals sampled from across the introduced ranges), and comprehensive sampling of potential source populations (74 native, 29 introduced *A. sagrei* populations and 40 native *A. cristatellus* populations).

5.1.2 Genetic processes: diversity and founder effects

The findings of this thesis do not support the association between low genetic diversity (a founder effect) and establishment success that has often been referred to as the paradox of invasion biology (Sakai *et al.* 2001; Allendorf & Lunquist 2003). Instead,

the work suggests that establishment success is associated with the presence of genetic diversity. The population of *A.sagrei* recently introduced to St. Vincent showed low establishment ability and no mtDNA haplotype diversity. The successfully established population of *A. cristatellus* on Dominica retained mtDNA haplotype diversity and microsatellite evidence indicated only a limited genetic founder effect. However, this cannot be rigorously tested in this thesis because mtDNA haplotype diversity cannot not accurately represent the population genetic diversity within the recently introduced *A. sagrei* population. MtDNA is subject to strong genetic drift because of its maternal and haploid mode of inheritance (Avise 1994) and thus, is often inadequate in representing introduced population genetic diversity. Microsatellite markers which assay variation at a population level were applied to *A. cristatellus* populations, however, there is a lack of replicated microsatellite genetic analyses, for reasons given in the above sections (i.e. species-specificity of microsatellite primers and a lack of replicated colonisations in this study).

Recent investigations have similarly revealed limited genetic founder effects in successful invasions by a range of taxa (including one of the two study species in this thesis, *A. sagrei*, Kolbe *et al.* 2004). The source of the genetic diversity uncovered by these studies can often be attributed to between-population genetic diversity in source ranges, i.e. genetic admixture resulting from multiple colonisations from different geographical areas of the native range (Barrett & Husband 1990; Barbaresi *et al.* 2003; Genton *et al.* 2005; Therriault *et al.* 2005; Dlugosch & Parker 2007). In contrast, this study demonstrates that the genetic diversity in a single founding propagule can originate from within-native population genetic diversity (microsatellite analysis revealed a limited founder effect in introduced *A. cristatellus*). Thus, the work presented here shows that genetic founder effects can be ameliorated in introduced populations if source populations contain high within-population genetic variability, for example, natural *Anolis* populations (multiple mtDNA haplotypes within admixed *A. roquet* populations, Thorpe *et al., submitted*; *A. sagrei* from Cuba up to 8% pairwise mtDNA sequence divergence within populations [mean = 1.7% across all Cuban populations] Kolbe *et al.* 2004).

The significance of the transfer of within-population genetic diversity in native ranges for introduced population genetic diversity has not previously been explored in invasion

studies. This is probably because for the majority of studies that report limited genetic founder effects, multiple introductions are either known or likely to have occurred, thus, within-native population genetic contributions cannot be separated from among-native population contributions to introduced genetic diversity. Multiple introductions can be discounted from the *A. cristatellus* introduction, thus revealing the likely source of genetic diversity for this colonisation, and also exposing this potential source of diversity for other single propagule introductions.

Furthermore, this work demonstrates that the transfer of within-population genetic diversity is dependant on colonisation history (propagule size and origin). Very small founding propagules may show limited or no haplotype diversity, despite high within-source population genetic diversity. The strong genetic founder effect revealed by mtDNA in St. Vincent *A. sagrei* is a likely to be a direct result of the historical influences of single introduction of a very small propagule from a highly restricted locality, despite high genetic diversity within the source population in Tampa, Florida. In summary, this work suggests that for introductions that are in early colonisation stages (less than *c.* 10 generations since import) genetic diversity is likely to be low because of limited opportunity for multiple introductions and associated genetic admixture, however, moderate propagule size and high within-native range genetic diversity can ameliorate this effect.

An additional source of genetic diversity in *Anolis* may result from life-history traits, and environmental influences on these traits. The ability of squamate females to store sperm from multiple mating events (Fox 1963; Conner & Crews 1980), together with the high fecundity of *Anolis*, may provide an important mechanism of haplotype transfer from native ranges, minimising genetic founder effects, provided that within-population genetic diversity is high in source populations. Rapid population growth (e.g. via high fecundity) enables the rapid establishment of self-sustaining populations, minimising the number of generations at small population size thereby largely evading the negative effects of genetic drift. Population growth rates can be affected by other factors, such as environmental disturbance. Disturbed, human influenced and /or fragmented habitats are commonly affected by introduced species because they generally promote spread and penetration. Native predators or competitors of introduced species may have abandoned disturbed habitat if it is sub-optimal for example, in resource availability or

predation risk, resulting in vacant niches that non-natives may occupy (Elton 1958; Hobbs & Huenneke 1992, although see Collins *et al.* 2007) enabling population expansion. Thus, life-history traits, and the strength of environmental influences on them, may be major contributors to the observed microsatellite genetic diversity recorded from introduced Dominican *A. cristatellus* populations. For *A. sagrei*, low initial propagule pressure may have obviated the genetic benefits of these traits. The findings of this thesis are thus consistent with the predictions of Nei *et al.* (1975) and Carson (1990), that losses of genetic diversity during an introduction may be modest, particularly if the bottleneck is short-lived, population growth is rapid, or the founding propagule is moderate or large.

5.1.3 Evidence of and potential for adaptive responses

Since the early classical studies on invasive exotic plants (Baker and Stebbins 1965; Baker 1974; Jain & Martins 1979), it has been recognised that introduced species often have significant amounts of evolutionary potential. The ability of colonising species to establish, proliferate and become invasive may be in part, an artefact of populations possessing genetic diversity with which to respond to local, novel selective pressures (Reznick & Ghalambor 2001; Lee 2002; Lambrinos 2004). Adaptive responses have been documented in a range of introduced species (Klepaker 1993; Huey *et al.* 2000; Costa-Pierce 2003; Rasner *et al.* 2004; Yeh 2004; Ayllon *et al.* 2006). However, in many studies, potentially adaptive or genetically-based traits are often compared between native and introduced populations, rather than between introduced populations (although see Johnston & Selander 1971; Carroll & Boyd 1992; Maron *et al.* 2004; Phillips *et al.* 2006). Differences reported from these comparisons may reflect shifts in phenotype due to genetic restructuring and drift (Templeton 1980; Carson & Templeton 1984). In contrast, differences in genetically-based traits between introduced populations may indicate an adaptive rather than historical explanation for the phenotypic variation. This association is only valid if the historical roles of insitu lineage sorting and drift arising from founder effects can be excluded as influencing factors.

This study reports altitudinal variation in (genetically controlled) scalation characters between introduced *A. cristatellus* populations on Dominica. MtDNA analyses eliminated founder effects and geographical segregation of haplotypes as explanations for the difference, indicating that selection for environmental conditions determines the altitudinal pattern of variation. This work supports a positive association between propagule size, genetic variability, potential for adaptive response and probability of establishment success, which has been suggested elsewhere (Lockwood *et al.* 2005). A moderate propagule size and limited founder effect indicate genetic diversity in introduced populations, suggesting a potential for adaptive response. Although estimates of genetic diversity and founder effects are based on neutral loci, they can indicate genome-wide genetic diversity, some of which is important for the response to selection (Fisher 1930; Reznick *et al.* 1997).

This is one of only a few studies to provide strong evidence of adaptive responses to environmental heterogeneity within in an introduced species' new range. Other such studies include adaptive differentiation in body proportions of house sparrows, *Passer domesticus* across the introduced North American range after introduction approximately 150 years ago (Johnston & Selander 1971) and a cline in flowering time related to climatic variability across the range of two *Solidago* (Asteraceae) species introduced to North America from Europe approximately 250 years ago (Weber & Schmid 1998). This study is also of value because it demonstrates a rate of adaptive change (within less than *c.* ten generations) which is in excess of that shown in the vast majority of invasive evolution studies (e.g. up to 150 generations in the soapberry bug *Jadera haematoloma*, Carroll & Boyd 1992), and in the order of that found in a few recent studies (e.g. less than 13 generations in sockeye salmon *Oncorhynchus nerka*, Hendry *et al.* 2000; 12-15 generations in St. Johns Wort *Hypericum perforatum,* Maron *et al.* 2004). Life-history characteristics and the ability of species to disperse can substantially influence how quickly adaptation can form and spread. Species which disperse rapidly, which includes most *Anolis spp.*, are more likely to encounter environmental heterogeneity across the occupied range, and thus be exposed to more variable selection pressures. The high dispersal ability of *A. cristatellus* has probably facilitated the expression of adaptive responses through exposure to varied environmental selective forces.

Phenotypic plasticity has long been acknowledged as a trait facilitating establishment and spread by invasive species (Marshall & Jain 1968; Rice & Mack 1991, Sexton *et al.* 2002). Environmental plasticity plays an important role in providing appropriate and rapid phenotypic responses to ecological pressures (Maron *et al.* 2004), and is increasingly recognised as being able to contribute to adaptive evolutionary responses (Ghalambor *et al.* 2007). Populations of *A. sagrei* experimentally introduced to small islands diverged significantly in phenotypic traits over a 10-14 year period (Losos *et al.* 1997). The direction of this morphological divergence was consistent with expectations from evolutionary diversification of *Anolis*, and the magnitude of difference in traits paralleled the magnitude of difference in habitat. This experimental work indicates that small propagules (5-10 individuals) can respond to environmental variability, via some phenotypic traits that have since been found to be predominantly under plastic control (Losos *et al.* 2000). Such plastic responses to environmental pressure may ultimately lead to the expression of genetically controlled adaptive traits.

Plasticity may place populations close enough to a new phenotypic optimum for directional selection to act (Ghalambor *et al.* 2007). The 'genes as followers' hypothesis (West-Eberhard 2003) proposes that adaptive responses can be initially established in a population via non-heritable plasticity, and later become genetically 'assimilated' (Baldwin 1896; Waddington 1942, 1953, 1956, 1959; Schmalhausen 1949). By this mechanism, such 'adaptive plasticity' may facilitate, or even speed up the process of adaptive evolution by reducing the cost of directional selection (e.g. Haldane 1957; Robinson & Dukas 1999; West-Eberhard 2003). The plasticity in scalation traits revealed in *A. cristatellus* populations varies with the same clines as genetically-based traits, and covaries with those previously described in the native anole (*A. oculatus*, Malhotra & Thorpe 1991b). These findings demonstrate that the roles of adaptation and plasticity may respond concurrently to environmental pressure, and suggest that environmental plasticity may represent a preliminary mechanism by which individuals may respond to novel environments before adaptive responses are feasible.

5.2 Wider implications

5.2.1 Utility of molecular methods for studying species introductions

The amount of neutral genetic variation measured by molecular markers may not be a reliable indicator of the amount of heritable variation for adaptive traits in the majority of natural populations (Frankham 1999; McKay & Latta 2002; Allendorf & Lundquist 2003). However, this variation is likely to be a reliable indicator of the potential for adaptive change in introduced species because a low genetic diversity in introduced populations relative to native populations indicates small effective population associated with a demographic bottleneck, which is expected to reduce the amount of variation at adaptive loci (Frankham 1999).

Mitochondrial sequence data has been widely used by evolutionary biologists to determine phylogenetic relationships, and timing, patterns and dynamics of adaptive and divergent events (Brooks & McLellan 1991; Harvey & Pagel 1991). This thesis demonstrates the value of mtDNA sequencing in reconstructing introduction history in the absence of detailed information about propagule size, propagule number and geographical origin. The minimum number of female founders can be revealed from the number of mitochondrial haplotypes, from which estimates of the initial founding population size may be approximated. The number of mtDNA haplotypes present can also provide an indication of the retention of haplotype diversity within introduced populations when compared to native populations (e.g. Puillandre *et al.* 2008), although do not accurately assay genetic diversity within populations (Avise 1994), especially recently introduced populations. In comparison to the evidence supplied by anecdotal reports, phylogenetic analyses can provide reliable estimates of the geographical origin of introduced haplotypes. This information can infer likely transportation routes and modes of importation, and the likelihood of multiple introduction events (and thus genetic admixture).

However, it is apparent from the findings of this thesis that caution must be applied when interpreting the results of phylogenetic analysis for geographical source identification. Haplotypes present in introduced populations may be absent from those identified in native ranges. For recent introductions, it is improbable that this absence can be explained by drift alone within such a timescale, and similarly unlikely that the same haplotype has been formed in the invasive range. The work presented in chapter 4 of this thesis suggests that haplotypes may be overlooked even by thorough sampling

regimes if there are multiple haplotypes within native populations, some of which are at low frequency. This finding recommends thorough assessments of within-native population genetic diversity and prudence in the interpretation of mtDNA analyses for identifying source populations. Dense within-population sampling of the likely geographical source area identified by preliminary phylogenetic reconstruction, may provide more reliable estimations of source populations in such cases. However, it is recognised that absolute certainty of the haplotype composition of native populations cannot be attained, and for the vast majority of cases, such high sampling effort is impractical. The intensity and strategy for sampling should be considered separately in each case, and provide a level of definition appropriate to within population genetic diversities.

Nuclear molecular markers generally retain variation for a longer period of time than mtDNA based methods (Neigel & Avise 1986; Villablanca *et al.* 1998). The use of fine-scale genetic markers, e.g. microsatellites, provide answers to a different set of questions based on the origins of genetic variation and the extent of genetic founder effects. The microsatellite evidence for *A. cristatellus* in Dominica demonstrates that analyses using population genetic markers can provide useful estimates of the amount and origin of genetic variation in introduced populations, which in turn indicates the potential for introduced populations to adapt to the new environment. Whilst many studies have used either mtDNA or microsatellites separately (reviewed in Puillandre *et al.* 2008, although see Grapputo *et al.* 2005; Villablanca *et al.* 1998), this thesis shows that a combined approach can prove cost and time effective in successfully determining the source population(s) for assaying population genetic variation. The value of linking molecular analyses with experimental common garden studies, to provide an explanation behind the observed variation in introduced species phenotype is shown in this thesis (see also Weber & Schmid 1998; Quinn *et al.* 2000). Analysing the distribution of mtDNA haplotypes in relation to the distribution of phenotypic traits within introduced ranges can reveal a historical (lineage sorting) explanation for the pattern of phenotypic trait distribution. Accurate results are dependant on a comprehensive and structured sampling approach, without which much information may be lost.

5.2.2 Relevance for colonisations and conservation

For already established invasive species, understanding population biology and genetics on a case-by-case basis may be unnecessary, time-consuming and may not even be helpful for their management and control. Furthermore, in many cases the best management strategy to reduce the risk of establishment by a recently introduced propagule of non-natives is to eliminate it before it has time to become abundant, widespread and evolve adaptations that may allow it to out-compete native species (Lambrinos 2004). Nonetheless, progress in understanding the genetics and evolution of introduced species can be helpful in identifying those that have the potential to become invasive, in predicting the potential for invasive species to evolve responses to management practices, and in the development of management policy.

The findings of this thesis provide important contributions to elucidating the predictors of establishment success and their interactive roles in colonisation processes. This information can be used to help predict the invasive potential of introductions, prioritise management efforts and select appropriate control efforts. Additionally, these findings may be extrapolated to provide predictions for the fate of populations that have similar historical characteristics to colonising propagules, such as small populations that have recently been isolated, for example, isolated populations of Blanding's turtles (*Emydoidea blandingii*) in the Greater Chicago Metropolitan area (Rubin *et al.* 2001) and a small population of fin whales (*Balaenoptera physalus*) in the Sea of Cortez, México (Bérubé *et al.* 2002).

The historical and genetic characterisation of both introduced and native populations can provide insights into the likelihood of establishment success. This study suggests that propagule size may be a simple, practical indicator to predict early-stage establishment success. The associated indication is that overcoming demographic stochasticity and the negative genetic correlates of small propagule size is important during early colonisation, and can negate the requirement for multiple introductions. This is an important revelation in invasion biology. Previously, multiple introductions were thought to have been a major influence in predicting establishment potential, by contributing to genetic diversity (Kolbe *et al.* 2004) and demographic pressure. However, the work presented in this thesis suggests that even relatively subtle increases in propagule size can positively influence establishment success. Thus, although

minimal or no conservation effort may suffice for potentially invasive importations involving very small propagules, for those of moderate propagule size, disproportionately more management attention may be required.

This thesis suggests that the establishment success of moderate-sized, single propagules can be augmented by life-history characteristics such as high fecundity, together with environmental conditions and the strength and direction of biotic interactions (e.g. the occupation of disturbed habitat, and low inter-specific competition) which may promote rapid population growth. Recognition of the influence of these factors on establishment ability in new colonisations, is an important step in identifying the potential for colonisations to become invasions. Such predictions may be of application in the control of planned introductions (for example in horticulture and biological control), by managing introduced populations for lower invasive potential (Barrett 1992).

The evidence presented here is consistent with other recent studies suggesting that the founder effect is not as ubiquitous as previously considered in colonisations (e.g. Barbaresi *et al.* 2003; Dlugosch & Parker 2007). This recent, continuing trend recommends the re-evaluation of invasion genetic theory and suggests the requirement for the revision of some invasive management strategies. For example, the genetic structure of introduced populations has been shown to affect the efficacy of management strategies (Burdon & Marshall 1981). In sexually reproducing weeds, greater genetic variation apparently allows more rapid adaptive evolution and escape from the biological control agents than asexually reproducing weeds (Van Driesche & Bellows 1996). Understanding the implications of high, or moderately high genetic diversity and its impact on adaptive responses, is therefore vital in selecting appropriate control methods for invasive populations.

Furthermore, the origin of this genetic diversity has important implications for management practice. This work provides some of the first evidence that naturally high genetic diversity within source populations can ameliorate genetic founder effects for the introduced population. Thus, even moderately-sized single propagules may contain sufficient genetic diversity to successfully establish, provided that within-source population diversity is high. This finding highlights the importance of revealing native range source populations and population genetic tools to predict establishment success.

It recommends that naturally high within-population genetic diversity may be an important 'invasive trait' although it may only substantially contribute to invasion success in combination with other factors. Nonetheless, increased growth in anthropogenic transportation, which can negate the need for high dispersal capacity and introduce single propagules into novel environments, suggests that within-native population genetic diversity could become a progressively more significant predictor of establishment potential.

Population genetic information about known invasive species should be managed, centralised and made available so that in the event of new colonisations, this information may be able to help predict establishment success, and therefore the need for management strategy. Online databases such as GenBank have subatantially contributed to the high accessibility of such information. Because this study shows that the transfer of within-population genetic diversity is also dependant on colonisation history (propagule size and origin), this information is also required if an effective management approach is to be adopted. Since multiple introductions often significantly alter the genetic and demographic properties of an invasion event, investigations centred around within-population genetic diversity may be more valuable in predicting the success of colonisations that are known to originate from single importations.

The interactions between genetic, ecological and evolutionary processes during the early stages of invasions are important in determining introduction success. For *A. cristatellus*, retention of genetic diversity from source populations is likely to have facilitated a rapid evolutionary response (within *c.* ten generations) to varying environmental selective forces in introduced ranges. This rapid, adaptive response to variation in selective pressure within introduced ranges is an important and infrequent observation and suggests that a similar response may possible in other recent introductions. Understanding the potential for introduced species to respond rapidly to environmental variation not only between source and introduced ranges, but within introduced ranges is important for predicting establishment potential. Whilst differences in phenotypic traits between source and introduced populations may be due to genetic drift, it is likely that genetic diversity within colonising populations is vital for adaptive responses to environmental selection pressures that vary within introduced ranges.

An understanding of population genetics may help predict the potential for populations of invasive species to evolve in new environments, or in response to management practices (for example, the evolution of resistance to herbicides or biological control agents, Barrett 1992; Van Driesche & Bellows 1996). This association has implications for conservation management. For example, following this rationale, it may be extrapolated that levels of genetic diversity indicated by molecular analyses can suggest the potential for small, bottlenecked populations to re-colonise environmentally heterogeneous areas. Additionally, knowledge of the mating systems and life-history characteristics of introduced species can provide insights into mechanisms for the transfer of genetic material and thus, the ability of populations to adapt. For example, plant species with both vegetative and sexual reproduction (Huenneke & Vitousek 1990) and species that are able to store sperm from multiple inseminations (e.g. insects, Simberloff 1989, and squamates, Fox 1963; Conner & Crews 1980; Whittier & Limpus 1996) may be able to establish successful populations that can respond to environmental pressure.

The speed at which introduced species can locally adapt, relative to native competitors or predators, can have important implications for establishment success. A rapid rate of adaptation, which may be influenced by dispersal ability and plasticity, can increase population and range expansion during introductions (Lambrinos 2004), thus determining which species have the potential to evolve rapidly can predict invasion potential. The speed at which introduced species can locally adapt, relative to adaptive shifts in native taxa responding to the introduced species may also be important in cases where there are strong inter-specific interactions associated with colonisation (Lambrinos 2004). For example, a shift in herbivore predation by the native weevil (*Euhrychiopsis lecontei*) has been associated with large declines in introduced populations of the Eurasian watermilfoil (*Myriophyllum spicatum*), which has been introduced to North American lakes and ponds (Sheldon & Creed 1995). This thesis has demonstrated that the roles of adaptation and plasticity may respond concurrently to environmental pressure, and suggest that environmental plasticity may represent a preliminary mechanism by which individuals may respond to novel environments before adaptive responses are feasible. Understanding that plasticity can play a key role in facilitating establishment is important for the targeting of conservation effort.

To identify factors that contribute to establishment success, it is important to consider both why some introductions succeed, and why others may fail. This thesis fulfils this criterion by including two ecologically similar, yet differently successful island colonisations by Caribbean *Anolis*. This work supports a positive association between propagule size, genetic variability, the potential for adaptive response and probability of establishment success. It demonstrates that this complex system of interacting factors can be influenced by life-history characteristics, environmental plasticity and external influences of environmental heterogeneity and biotic interactions. Importantly, this thesis recommends that conservation managers must recognize that a single propagule of moderate size may contain sufficient genetic diversity to successfully establish and respond to novel selective forces in their new ranges. Accordingly, extrapolating from the findings of the *A. sagrei* study, very small propagule size is predicted to hold low potential for establishment success. The understanding of the interactions between historical, genetic and evolutionary processes has a broad application for control and management of introduced species. For example, unless nations can demonstrate that the importation of new genetic material poses a credible risk, they are unable to regulate the importation of established pests under World Trade Organisation agreements (Campbell 2001). However, there is not yet a comprehensive understanding of the genetic structure of most introduced populations or framework to predict ecological and economic risk associated with the introduction of new genetic material. Only an increase in studies that investigate the genetic and evolutionary processes involved in colonisations can shed light on this increasingly recognised, often detrimental influence on natural ecosystems.

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APPENDIX I

Position of major sampling localities used for this study.

Localities sampled for microsatellite and common garden experiments

Locality name	North			West		
	degrees	minutes	seconds	degrees	minutes	seconds
Dominica						
Roger Low	15°	20'	0.1''	61°	23'	23.4''
Morne Daniel Low	15°	19'	40.6''	61°	23'	32''
Bellevue Low	15°	18'	31.6''	61°	23'	4''
Botanical Gardens Low	15°	17'	58.5''	61°	22'	57.6''
Roger High	15°	20'	23.8''	61°	23'	4.2''
Morne Daniel High	15°	19'	20.3''	61°	23'	13.7''
Bellevue High	15°	18'	36.9''	61°	22'	37.2''
Botanical Gardens High	15°	17'	53.6''	61°	22'	46.1''
Jimmet	15°	22'	21.5''	61°	24'	19.1''
Bateaux	15°	15'	0''	61°	22'	28.2''
Puerto Rico						
Cambalache	18°	27'	58.3''	66°	32'	54.5''
Laguna Tortuguero	18°	27'	10.6''	66°	25'	13.1''
Manati	18°	23'	8.5''	66°	27'	36.4''
Bosque Rio Abajo	18°	19'	38.6''	66°	40'	39.9''
Ciales	18°	20'	36.9''	66°	26'	9.3''
Orocovis	18°	12'	11.5''	66°	24'	44.7''
Tupi's House	18°	30'	5.5''	67°	8'	40.3''
St. Vincent						
Campden Park Dock	13°	10'	16.9''	61°	14'	55.6''

Localities sampled for mitochondrial DNA sequencing

Locality code	North			West		
	degrees	minutes	seconds	degrees	minutes	seconds
PRC10	18°	15'	4.7''	66°	50'	46.7''
PRC2	18°	27'	31.9''	66°	48'	28.8''
PRC4	18°	24'	20.7''	66°	47'	55.6''
PRC6	18°	20'	34.3''	66°	49'	10.2''
PRC8	18°	17'	44.3''	66°	48'	25.5''
PRE10	18°	13'	7.8''	66°	46'	5.3''
PRE2	18°	27'	52.7''	66°	44'	50.3''
PRE3	18°	25'	41.8''	66°	44'	53.1''
PRE4	18°	23'	30.5''	66°	45'	2.1''
PRE5	18°	22'	13.5''	66°	45'	0.3''
PRE6	18°	20'	59.3''	66°	45'	8''
PRE7	18°	17'	55.3''	66°	44'	20.2''
PRE8	18°	16'	46.2''	66°	45'	13.7''
PRF3	18°	26'	28.1''	66°	42'	58.3''
PRF4	18°	23'	38.1''	66°	43'	5.6''
PRF5	18°	22'	33''	66°	42'	25.1''
PRF6	18°	19'	43.7''	66°	42'	30.2''
PRF7	18°	17'	38.7''	66°	43'	8.1''
PRF8	18°	16'	22.9''	66°	42'	59.1''
PRG10	18°	14'	7.5''	66°	40'	42.9''
PRG2	18°	28'	2.8''	66°	42'	16.2''
PRG3	18°	25'	5.3''	66°	40'	59.2''
PRG4	18°	22'	50.6''	66°	41'	10.9''
PRG5	18°	21'	48.4''	66°	41'	34.4''
PRG6	18°	19'	38.6''	66°	40'	39.9''
PRG7	18°	18'	4.8''	66°	42'	50.3''
PRG8	18°	15'	47.6''	66°	41'	27.6''
PRH2	18°	27'	26.7''	66°	39'	5.5''
PRH3	18°	25'	44.1''	66°	38'	58.4''
PRH4	18°	24'	5.1''	66°	40'	9.5''
PRH5	18°	22'	31''	66°	39'	38''
PRH6	18°	20'	23.6''	66°	40'	21.8''
PRH7	18°	17'	43.2''	66°	38'	32.8''
PRH8	18°	16'	38.4''	66°	39'	1''
PRI10	18°	13'	8.4''	66°	38'	9.2''
PRI2	18°	27'	37.8''	66°	37'	24.2''
PRI3	18°	25'	41.7''	66°	37'	32''
PRI4	18°	24'	24.1''	66°	38'	16.7''
PRI5	18°	23'	8.9''	66°	38'	2.2''
PRI6	18°	19'	22.7''	66°	36'	16.4''
PRI8	18°	16'	2.6''	66°	39'	5.6''
PRJ2	18°	27'	11.8''	66°	35'	50.7''
PRJ3	18°	25'	52.1''	66°	34'	51.2''
PRJ4	18°	24'	20.5''	66°	35'	46.7''
PRJ5	18°	22'	36.8''	66°	35'	33.2''

PRJ6	18°	20'	36.7''	66°	35'	10.9''
PRK10	18°	13'	11''	66°	36'	46.8''
PRK2	18°	27'	58.3''	66°	32'	54.5''
PRK3	18°	25'	30''	66°	34'	12.7''
PRK4	18°	24'	13.2''	66°	33'	15.4''
PRK5	18°	22'	57.9''	66°	33'	47.3''
PRK6	18°	20'	52.5''	66°	34'	24.9''
PRK8	18°	15'	24.7''	66°	34'	28.8''
PRL2	18°	27'	47.2''	66°	31'	58.2''
PRL3	18°	25'	36.2''	66°	31'	38.1''
PRL4	18°	25'	4''	66°	31'	22.8''
PRL5	18°	21'	49.1''	66°	33'	35''
PRL6	18°	21'	10.8''	66°	32'	39.1''
PRM10	18°	13'	30.6''	66°	30'	4.9''
PRM2	18°	27'	42.1''	66°	29'	36''
PRM3	18°	25'	49.7''	66°	29'	37.9''
PRM4	18°	24'	9.9''	66°	29'	31.9''
PRM5	18°	22'	29.6''	66°	30'	27.1''
PRM6	18°	18'	32.4''	66°	31'	15.6''
PRM8	18°	16'	33.3''	66°	28'	45.5''
PRN3	18°	25'	54.3''	66°	27'	53.1''
PRN4	18°	23'	8.5''	66°	27'	36.4''
PRN5	18°	21'	38''	66°	27'	57.1''
PRO10	18°	12	11.5	66°	24	44.7
PRO2	18°	27	10.6	66°	25	13.1
PRO3	18°	25	52.5	66°	25	59.4
PRO5	18°	23	5.7	66°	25	28.1
PRO6	18°	20	36.9	66°	26	9.3
PRO8	18°	15	59	66°	26	20.9
PRQ10	18°	13	4.9	66°	22	27.5
PRQ2	18°	27	38.8	66°	21	54.5
PRQ4	18°	23	57.7	66°	22	20.6
PRQ6	18°	20	19.5	66°	22	32.5
PRQ8	18°	17	6	66°	24	16

S5.

Haplotypes isolated from individuals sampled for this study in Puerto Rico, and localities from which individuals were sampled. Frequency of haplotypes genotyped at localities is also shown. Due to failed amplification and low quality of gene products, only 111 of the 159 sampled individuals were reliably genotyped.

Haplotype	Clade	Localities/no. of individuals from which haplotype was isolated							
		Locality	Frequency	Locality	Frequency	Locality	Frequency	Locality	Frequency
H-PR01	Central West PR	Cambalache	-	PRJ2	1	PRJ2	1		
H-PR02	Central West PR	PRO4	1	PRO3	1				
H-PR03	Central West PR	PRN4	1	PRM2	1	PRM4	1	PRM6	1
H-PR04	Central West PR	PRN5	1	PRM4	1				
H-PR05	Central West PR	PRK2	1	PRL2	1	PRL3	2		
H-PR06	Central West PR	PRO2	2	PRO3	1	PRM2	1		
H-PR07	Central East PR	Morovis	-	PRK5	1				
H-PR08	Central East PR	PRQ4	1						
H-PR09	Central East PR	PRM10	2	PRM8	1				

Haplotype	Clade	Localities/no. of individuals from which haplotype was isolated							
		Locality	Frequency	Locality	Frequency	Locality	Frequency	Locality	Frequency
H-PR10	Central East PR	PRM6	1	PRJ6	1	PRL4	1	PRN4	1
H-PR11	Central East PR	PRM8	1	PRL6	1				
H-PR12	Central East PR	PRQ10	1	PRN5	1				
H-PR13	Central East PR	PRO4	1						
H-PR14	Central West PR	Cambalache	-	PRI2	1				
H-PR15	Central West PR	Cambalache	-	PRK2	1	PRI2	1		
H-PR16	Central West PR	Utuado	-	PRK10	2	PRG10	1		
H-PR17	Central West PR	PRC8	2	PRC10	2	PRE10	2	PRG10	1
H-PR18	Central West PR	PRK4	2	PRK3	1	PRI3	1		
H-PR19	Central West PR	PRI3	1	PRJ4	1	PRK3	1		
H-PR20	Central West PR	PRH5	1	PRG4	2	PRI4	1	PRH4	2
H-PR21	Central West PR	PRL6	1	PRI6	1	PRK6	2	PRL5	2
H-PR22	Central East PR	Morovis	-						

Haplotype	Clade	Localities/no. of individuals from which haplotype was isolated							
		Locality	Frequency	Locality	Frequency	Locality	Frequency	Locality	Frequency
H-PR23	Central East PR	PRQ4	1						
H-PR24	West PR	PRC6	1	PRF6	1	PRE5	1		
H-PR25	West PR	PRC2	2	PRE2	2				
H-PR26	West PR	PRC4	1	PRC6	1	PRE4	1	PRE3	1
H-PR27	West PR	PRE4	1	PRE3	1	PRG3	1	PRG5	1
H-PR28	West PR	PRE5	1	PRE7	1	PRF5	2	PRF6	1
H-PR29	West PR	PRG6	1	PRF7	1	PRG7	1	PRG8	2
H-PR30	West PR	PRF4	2	PRF3	2	PRG3	1	PRG2	1
H-PR31	West PR	PRG5	1	PRG6	1				

Haplotype distribution among sites in Dominica (no. of individuals sequenced, n=98).

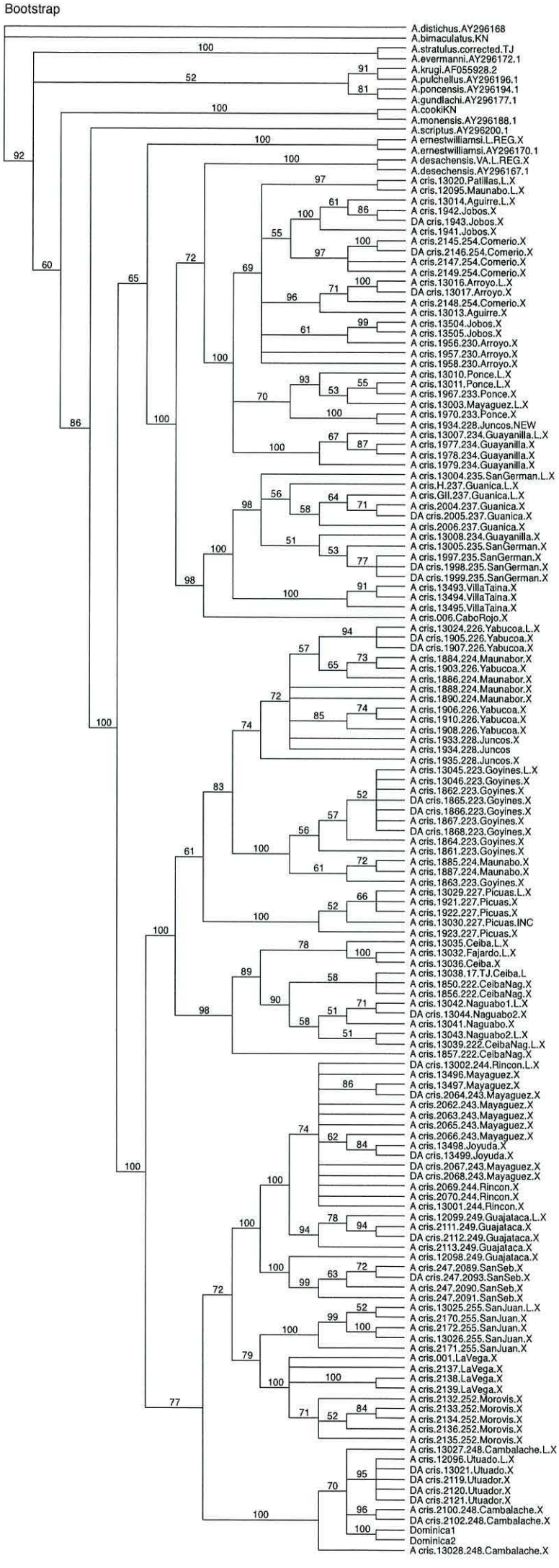
Locality	DO1	DO2	DO3	DO4	DO5	DO6	DO7	DO8	DO9	D10
Haplotype										
H-D1	9	0	1	3	0	0	7	4	0	0
H-D2	0	6	4	1	2	1	0	1	1	0
H-D3	0	0	1	0	0	0	0	0	0	0
H-D4	0	8	1	6	6	1	0	1	16	3
H-D5	0	0	2	3	0	5	0	1	0	0
H-D6	0	0	0	0	0	1	0	0	0	0
H-D7	0	0	0	0	0	0	0	3	0	0

Dominica Range Limits (from February 2005)

Month/year	Year	Limit	Locality name	North degrees	minutes	seconds	West degrees	minutes	seconds
Feb-05	2005	N	JIMMET	15°	22'	21.5''	61°	24'	19.1''
Feb-05		E	NLIMIT0205	15°	20'	22.6''	61°	22'	58.3''
Feb-05		S	BATEAUX	15°	15'	0''	61°	22'	28.2''
Feb-06	2006	N	NLIMIT0206	15°	20'	28.3''	61°	22'	41.6''
Jun-06		N	JIMMET06	15°	22'	45.1''	61°	24'	30.6''
Jun-06		E	EMSALL06	15°	18'	1.1''	61°	22'	34.8''
Jun-06		E	ST AROM	15°	18'	26.4''	61°	22'	19.3''
Jun-06		S	LOUBIER06	15°	16'	32.6''	61°	22'	15.5''
Jun-07	2007	N	JUN N 07	15°	22'	55.6''	61°	24'	39.9''
Jun-07		E	JUN07	15°	20'	25.9''	61°	22'	55.2''
Jun-07		S	BATALI07	15°	27'	13.3''	61°	26'	48.7''
Feb-08	2008	N	NFEB08	15°	23'	15.8''	61°	24'	55.1''
Feb-08		E	E FEB08	15°	20'	28.9''	61°	22'	50.8''
Feb-08		S	FEB08	15°	15'	20.3''	61°	22'	32.9''

APPENDIX II

MtDNA phylogeny of Puerto Rican *A. cristatellus*: unpublished data from Rich Glor


Bootstrap
A.distichus.AY296168
A.bimaculatus.KN
A.stratulus.corrected.TJ
A.evermanni.AY296172.1
A.krugi.AF055928.2
A.pulchellus.AY296196.1
A.poncensis.AY296194.1
A.gundlachi.AY296177.1
A.cookiKN
A.monensis.AY296188.1
A.scriptus.AY296200.1
A ernestwilliamsi.L.REG.X
A.ernestwilliamsi.AY296170.1
A desachensis.VA.L.REG.X
A.desechensis.AY296167.1
A cris.13020.Patillas.L.X
A cris.12095.Maunabo.L.X
A cris.13014.Aguirre.L.X
A cris.1942.Jobos.X
DA cris.1943.Jobos.X
A cris.1941.Jobos.X
A cris.2145.254.Comerio.X
DA cris.2146.254.Comerio.X
A cris.2147.254.Comerio.X
A cris.2149.254.Comerio.X
A cris.13016.Arroyo.L.X
DA cris.13017.Arroyo.X
A cris.2148.254.Comerio.X
A cris.13013.Aguirre.X
A cris.13504.Jobos.X
A cris.13505.Jobos.X
A cris.1956.230.Arroyo.X
A cris.1957.230.Arroyo.X
A cris.1958.230.Arroyo.X
A cris.13010.Ponce.L.X
A cris.13011.Ponce.L.X
A cris.1967.233.Ponce.X
A cris.13003.Mayaguez.L.X
A cris.1970.233.Ponce.X
A cris.1934.228.Juncos.NEW
A cris.13007.234.Guayanilla.L.X
A cris.1977.234.Guayanilla.X
A cris.1978.234.Guayanilla.X
A cris.1979.234.Guayanilla.X
A cris.13004.235.SanGerman.L.X
A cris.H.237.Guanica.L.X
A cris.GII.237.Guanica.L.X
A cris.2004.237.Guanica.X
DA cris.2005.237.Guanica.X
A cris.2006.237.Guanica.X
A cris.13008.234.Guayanilla.X
A cris.13005.235.SanGerman.X
A cris.1997.235.SanGerman.X
DA cris.1998.235.SanGerman.X
DA cris.1999.235.SanGerman.X
A cris.13493.VillaTaina.X
A cris.13494.VillaTaina.X
A cris.13495.VillaTaina.X
A cris.006.CaboRojo.X
A cris.13024.226.Yabucoa.L.X
DA cris.1905.226.Yabucoa.X
DA cris.1907.226.Yabucoa.X
A cris.1884.224.Maunabor.X
A cris.1903.226.Yabucoa.X
A cris.1886.224.Maunabor.X
A cris.1888.224.Maunabor.X
A cris.1890.224.Maunabor.X
A cris.1906.226.Yabucoa.X
A cris.1910.226.Yabucoa.X
A cris.1908.226.Yabucoa.X
A cris.1933.228.Juncos.X
A cris.1934.228.Juncos
A cris.1935.228.Juncos.X
A cris.13045.223.Goyines.L.X
A cris.13046.223.Goyines.X
A cris.1862.223.Goyines.X
DA cris.1865.223.Goyines.X
DA cris.1866.223.Goyines.X
A cris.1867.223.Goyines.X
DA cris.1868.223.Goyines.X
A cris.1864.223.Goyines.X
A cris.1861.223.Goyines.X
A cris.1885.224.Maunabo.X
A cris.1887.224.Maunabo.X
A cris.1863.223.Goyines.X
A cris.13029.227.Picuas.L.X
A cris.1921.227.Picuas.X
A cris.1922.227.Picuas.X
A cris.13030.227.Picuas.INC
A cris.1923.227.Picuas.X
A cris.13035.Ceiba.L.X
A cris.13032.Fajardo.L.X
A cris.13036.Ceiba.X
A cris.13038.17.TJ.Ceiba.L
A cris.1850.222.CeibaNag.X
A cris.1856.222.CeibaNag.X
A cris.13042.Naguabo1.L.X
DA cris.13044.Naguabo2.X
A cris.13041.Naguabo.X
A cris.13043.Naguabo2.L.X
A cris.13039.222.CeibaNag.L.X
A cris.1857.222.CeibaNag.X
DA cris.13002.244.Rincon.L.X
A cris.13496.Mayaguez.X
A cris.13497.Mayaguez.X
DA cris.2064.243.Mayaguez.X
A cris.2062.243.Mayaguez.X
A cris.2063.243.Mayaguez.X
A cris.2065.243.Mayaguez.X
A cris.2066.243.Mayaguez.X
A cris.13498.Joyuda.X
DA cris.13499.Joyuda.X
DA cris.2067.243.Mayaguez.X
DA cris.2068.243.Mayaguez.X
A cris.2069.244.Rincon.X
A cris.2070.244.Rincon.X
A cris.13001.244.Rincon.X
A cris.12099.249.Guajataca.L.X
A cris.2111.249.Guajataca.X
DA cris.2112.249.Guajataca.X
A cris.2113.249.Guajataca.X
A cris.12098.249.Guajataca.X
A cris.247.2089.SanSeb.X
DA cris.247.2093.SanSeb.X
A cris.247.2090.SanSeb.X
A cris.247.2091.SanSeb.X
A cris.13025.255.SanJuan.L.X
A cris.2170.255.SanJuan.X
A cris.2172.255.SanJuan.X
A cris.13026.255.SanJuan.X
A cris.2171.255.SanJuan.X
A cris.001.LaVega.X
A cris.2137.LaVega.X
A cris.2138.LaVega.X
A cris.2139.LaVega.X
A cris.2132.252.Morovis.X
A cris.2133.252.Morovis.X
A cris.2134.252.Morovis.X
A cris.2136.252.Morovis.X
A cris.2135.252.Morovis.X
A cris.13027.248.Cambalache.L.X
A cris.12096.Utuado.L.X
DA cris.13021.Utuado.X
DA cris.2119.Utuador.X
DA cris.2120.Utuador.X
DA cris.2121.Utuador.X
A cris.2100.248.Cambalache.X
DA cris.2102.248.Cambalache.X
Dominica1
Dominica2
A cris.13028.248.Cambalache.X

APPENDIX III

GenBank accession numbers for Mitochondrial DNA (NADH2) sequences from samples collected and sequenced for this study.

Specimen Voucher	Accession number
EAL_C2	EU599051
EAL_C4	EU599052
EAL_C6	EU599053
EAL_C8	EU599054
EAL_E4	EU599055
EAL_E5	EU599056
EAL_F4	EU599057
EAL_G5	EU599058
EAL_G6	EU599059
EAL_H5	EU599060
EAL_I3	EU599061
EAL_K2	EU599062
EAL_K4	EU599063
EAL_L6	EU599064
EAL_M10	EU599065
EAL_M6	EU599066
EAL_M8	EU599067
EAL_N4	EU599068
EAL_N5	EU599069
EAL_O10	EU599070
EAL_O2	EU599071
EAL_O4	EU599072
EAL_O6	EU599073
EAL_Q4	EU599074
EAL_Q6	EU599075
EAL_BL07	EU599076
EAL_CAN02	EU599077
EAL_CAN03	EU599078
EAL_GH10	EU599079
EAL_MH05	EU599080
EAL_RL01	EU599081
EAL_ROS01	EU599082

APPENDIX IV

Microsatellite allele frequency data for *A. cristatellus* from Puerto Rico and Dominica sampled for this study.

Locus	Allele	PR01	PR02	PR03	PR04	PR05	PR06	PR07	DO1	DO2	DO3	DO4	DO5	DO6	DO7	DO8	DO9
CRIS 124	**161**	0.014	0.044	0.113	0.088	0.054	0.173	0.000	0.122	0.221	0.143	0.086	0.088	0.145	0.052	0.078	0.054
	163	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	165	0.000	0.000	0.000	0.059	0.014	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	167	0.056	0.132	0.032	0.250	0.054	0.096	0.015	0.068	0.147	0.100	0.190	0.088	0.145	0.207	0.188	0.107
	169	0.153	0.382	0.258	0.147	0.149	0.135	0.206	0.216	0.338	0.543	0.397	0.338	0.274	0.345	0.344	0.339
	171	0.236	0.088	0.129	0.029	0.108	0.019	0.221	0.027	0.088	0.086	0.121	0.088	0.161	0.138	0.078	0.214
	173	0.028	0.074	0.113	0.118	0.041	0.096	0.015	0.149	0.000	0.000	0.017	0.013	0.016	0.138	0.000	0.000
	175	0.153	0.147	0.065	0.000	0.081	0.019	0.162	0.162	0.074	0.014	0.017	0.100	0.016	0.017	0.016	0.018
	177	0.111	0.059	0.016	0.015	0.041	0.115	0.147	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.031	0.000
	179	0.083	0.044	0.065	0.059	0.081	0.173	0.088	0.054	0.029	0.000	0.000	0.038	0.016	0.017	0.000	0.089
	181	0.056	0.000	0.048	0.059	0.027	0.077	0.044	0.000	0.000	0.000	0.017	0.000	0.065	0.000	0.016	0.036
	183	0.083	0.000	0.016	0.044	0.122	0.000	0.000	0.000	0.029	0.000	0.000	0.113	0.016	0.000	0.000	0.000
	185	0.014	0.000	0.000	0.015	0.041	0.000	0.000	0.203	0.074	0.114	0.069	0.125	0.097	0.052	0.141	0.107
	187	0.000	0.015	0.065	0.015	0.068	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	189	0.000	0.000	0.016	0.088	0.000	0.038	0.103	0.000	0.000	0.000	0.034	0.000	0.016	0.017	0.000	0.018
	191	0.014	0.000	0.032	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	193	0.000	0.015	0.032	0.015	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.017	0.047	0.000
	195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.013	0.000	0.000	0.063	0.018
	197	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CRIS 128	**139**	0.028	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	141	0.000	0.015	0.000	0.000	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	143	0.000	0.015	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	145	0.014	0.000	0.032	0.015	0.054	0.058	0.000	0.014	0.000	0.000	0.034	0.000	0.000	0.000	0.016	0.000
	147	0.014	0.015	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Locus	Allele	PR01	PR02	PR03	PR04	PR05	PR06	PR07	DO1	DO2	DO3	DO4	DO5	DO6	DO7	DO8	DO9
CRIS 128	**149**	0.000	0.000	0.000	0.029	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(cont.)	**151**	0.111	0.118	0.129	0.088	0.095	0.077	0.368	0.230	0.074	0.143	0.017	0.025	0.016	0.086	0.063	0.107
	153	0.306	0.294	0.226	0.250	0.230	0.250	0.221	0.243	0.294	0.143	0.259	0.525	0.306	0.190	0.344	0.214
	155	0.208	0.206	0.355	0.221	0.203	0.385	0.279	0.054	0.206	0.157	0.362	0.263	0.339	0.207	0.359	0.286
	157	0.139	0.118	0.161	0.191	0.122	0.019	0.044	0.162	0.029	0.086	0.034	0.025	0.016	0.207	0.063	0.018
	159	0.153	0.103	0.032	0.074	0.095	0.000	0.015	0.135	0.250	0.271	0.259	0.138	0.226	0.241	0.109	0.196
	161	0.028	0.088	0.065	0.059	0.203	0.096	0.000	0.162	0.147	0.200	0.034	0.025	0.097	0.069	0.047	0.179
	163	0.000	0.000	0.000	0.015	0.000	0.077	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	165	0.000	0.015	0.000	0.029	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	167	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CRIS 136	**209**	0.014	0.029	0.016	0.118	0.000	0.000	0.000	0.081	0.000	0.000	0.017	0.000	0.000	0.086	0.047	0.000
	211	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	213	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.014	0.052	0.063	0.016	0.017	0.000	0.018
	217	0.042	0.015	0.065	0.176	0.014	0.250	0.029	0.095	0.015	0.029	0.000	0.063	0.000	0.034	0.016	0.018
	219	0.208	0.265	0.274	0.221	0.149	0.481	0.412	0.095	0.191	0.357	0.017	0.175	0.145	0.241	0.078	0.143
	221	0.361	0.426	0.177	0.015	0.405	0.077	0.279	0.338	0.412	0.457	0.586	0.575	0.500	0.517	0.688	0.446
	223	0.014	0.000	0.065	0.103	0.135	0.058	0.000	0.122	0.074	0.029	0.000	0.000	0.194	0.017	0.031	0.054
	225	0.153	0.000	0.032	0.029	0.027	0.019	0.235	0.000	0.147	0.029	0.138	0.113	0.065	0.017	0.078	0.214
	227	0.208	0.162	0.161	0.279	0.230	0.077	0.015	0.230	0.029	0.071	0.155	0.013	0.065	0.017	0.016	0.089
	229	0.000	0.044	0.081	0.000	0.027	0.000	0.000	0.041	0.118	0.014	0.017	0.000	0.016	0.052	0.047	0.018
	231	0.000	0.000	0.048	0.015	0.014	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	233	0.000	0.059	0.000	0.029	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	235	0.000	0.000	0.081	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CRIS 140	**292**	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	294	0.028	0.015	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
	296	0.014	0.044	0.081	0.000	0.081	0.077	0.324	0.054	0.000	0.029	0.034	0.075	0.000	0.052	0.016	0.000
	298	0.069	0.044	0.065	0.044	0.014	0.077	0.088	0.068	0.088	0.271	0.052	0.088	0.097	0.052	0.172	0.089

Locus	Allele	PR01	PR02	PR03	PR04	PR05	PR06	PR07	DO1	DO2	DO3	DO4	DO5	DO6	DO7	DO8	DO9
CRIS 140	**300**	0.083	0.176	0.129	0.206	0.284	0.115	0.103	0.041	0.000	0.014	0.000	0.013	0.065	0.052	0.047	0.125
(cont.)	**302**	0.097	0.118	0.081	0.059	0.027	0.115	0.088	0.108	0.147	0.014	0.103	0.300	0.081	0.155	0.094	0.125
	304	0.167	0.147	0.048	0.059	0.041	0.019	0.088	0.054	0.044	0.129	0.052	0.088	0.048	0.345	0.094	0.054
	306	0.083	0.074	0.177	0.015	0.230	0.058	0.000	0.176	0.118	0.071	0.276	0.088	0.145	0.052	0.094	0.018
	308	0.111	0.074	0.113	0.132	0.027	0.096	0.074	0.068	0.000	0.043	0.000	0.038	0.000	0.000	0.047	0.000
	310	0.083	0.088	0.065	0.162	0.041	0.058	0.132	0.135	0.074	0.057	0.103	0.038	0.048	0.017	0.016	0.107
	312	0.028	0.029	0.065	0.074	0.095	0.058	0.000	0.135	0.235	0.229	0.034	0.013	0.065	0.034	0.078	0.018
	314	0.083	0.015	0.016	0.015	0.068	0.038	0.015	0.054	0.074	0.086	0.241	0.150	0.113	0.121	0.156	0.196
	316	0.069	0.000	0.016	0.088	0.014	0.269	0.044	0.081	0.162	0.029	0.000	0.050	0.161	0.034	0.078	0.089
	318	0.000	0.000	0.016	0.044	0.041	0.019	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	320	0.083	0.162	0.129	0.044	0.027	0.000	0.000	0.027	0.029	0.029	0.103	0.050	0.113	0.086	0.109	0.179
	322	0.000	0.015	0.000	0.029	0.014	0.000	0.000	0.000	0.029	0.000	0.000	0.013	0.048	0.000	0.000	0.000
CRIS 22	**178**	0.042	0.000	0.048	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	182	0.000	0.000	0.000	0.000	0.000	0.000	0.147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	184	0.181	0.074	0.113	0.103	0.162	0.154	0.103	0.068	0.044	0.000	0.052	0.100	0.032	0.086	0.031	0.071
	186	0.014	0.029	0.000	0.000	0.014	0.077	0.000	0.230	0.118	0.171	0.034	0.125	0.081	0.017	0.047	0.018
	188	0.111	0.338	0.129	0.265	0.122	0.250	0.338	0.108	0.103	0.100	0.276	0.288	0.032	0.172	0.156	0.214
	190	0.153	0.074	0.194	0.338	0.230	0.231	0.015	0.311	0.250	0.186	0.190	0.075	0.226	0.138	0.172	0.286
	192	0.014	0.000	0.000	0.015	0.000	0.115	0.015	0.000	0.044	0.000	0.017	0.013	0.016	0.121	0.063	0.054
	194	0.444	0.132	0.129	0.044	0.041	0.077	0.176	0.122	0.044	0.000	0.000	0.000	0.048	0.086	0.016	0.054
	196	0.000	0.015	0.097	0.015	0.054	0.000	0.000	0.041	0.059	0.029	0.017	0.025	0.129	0.052	0.047	0.089
	198	0.014	0.029	0.145	0.118	0.243	0.038	0.162	0.068	0.162	0.114	0.190	0.125	0.177	0.052	0.250	0.036
	200	0.028	0.088	0.065	0.044	0.014	0.058	0.029	0.014	0.044	0.171	0.052	0.113	0.161	0.034	0.078	0.054
	202	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.014	0.029	0.014	0.017	0.000	0.000	0.017	0.016	0.054
	206	0.000	0.044	0.000	0.015	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.214	0.052	0.063	0.065	0.069	0.047	0.018
	214	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	216	0.000	0.000	0.000	0.015	0.027	0.000	0.000	0.000	0.000	0.000	0.069	0.013	0.016	0.086	0.031	0.018

Locus	Allele	PR01	PR02	PR03	PR04	PR05	PR06	PR07	DO1	DO2	DO3	DO4	DO5	DO6	DO7	DO8	DO9
CRIS 22	**220**	0.000	0.044	0.048	0.015	0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(cont.)	**222**	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.027	0.029	0.000	0.034	0.063	0.016	0.069	0.047	0.036
	224	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX V

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Weak founder effect signal in a recent introduction of Caribbean *Anolis*

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Abstract

Species introductions provide a rare opportunity to study rapid evolutionary and genetic processes in natural systems, often under novel environmental pressures. Few empirical studies have been able to characterize genetic founder effects associated with demographic bottlenecks at the earliest stages of species introductions. This study utilizes prior mitochondrial DNA information which identifies the putative source population for a recently established (*c.* 7 years between import and sampling) species introduction. We investigated the evidence for a founder effect in a highly successful introduction of a Puerto Rican *Anolis* species that has established itself on Dominica to the localized exclusion of the native, endemic anole. Five highly polymorphic microsatellite loci were used to explore the partitioning of genetic diversity within and between native source, native nonsource, and introduced populations of *Anolis cristatellus*. Group comparisons reveal significantly lower allelic richness and expected heterozygosity in introduced populations compared to native populations; however, tests for heterozygosity excess relative to allelic richness failed to provide consistent evidence for a founder effect within introduced populations. Significant levels of within-population genetic variation were present in both native and introduced populations. We suggest that aspects of the reproductive ecology of *Anolis* (high fecundity, sperm storage and multiple paternity) offer an important mechanism by which genetic variation may be maintained following demographic bottlenecks and founder events in some squamate taxa.

Keywords: *Anolis cristatellus*, founder effect, genetic variability, microsatellite DNA, sperm storage

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Introduction

Introduced species have long been recognized as having important economic and ecological effects (Elton 1958; Simberloff & Stiling 1996; Pimmental 2000; Sakai *et al.* 2001) and as such they have been much studied. More recently, their potential as model systems of evolutionary processes has been realized (Lambrinos 2004). The introduction of a small founder population into a new, non-native locale (often by anthropogenic means of transportation) offers a rare opportunity to study rapid evolutionary processes under something approaching natural conditions. Furthermore, because introductions often involve novel environments with different ecological challenges to those which the species has been previously exposed, they have been frequently associated with adaptive evolution (Rasner *et al.* 2004). Molecular genetic methods are a powerful tool in invasion studies, providing evidence to link introduced populations to their potential native range sources as well as exposing genetic differences between these areas, suggesting that evolutionary processes may have taken place (Cornuet & Luikart 1996). Identifying the source population is a significant step in the investigation of an invasion event, enabling reconstruction of past events leading up to the introduction (Williamson 1996) and exploration of the potential for appropriate control and management. Molecular data [e.g. mtDNA (mitochondrial DNA) sequence data] together with phylogeographical analysis may be used to elucidate the most probable source population in the native range (Hufbauer *et al.* 2004; J. Eales, R.S. Thorpe and A. Malhotra, unpublished data). Subsequent morphological

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and genetic comparison of the source and invasive populations using fine-scale nuclear markers (e.g. microsatellites) can provide detailed information about the amount of genetic variation introduced (Cornuet & Luikart 1996), the effects of genetic diversity on introduction success (Veltman *et al.* 1996), population fitness (Reed & Frankham 2003) and species ecology (Tsutsui *et al.* 2000; Tsutsui *et al.* 2001). In addition, the study of processes associated with known small founder populations and genetic bottlenecks provides an invaluable model that has relevance to the substantial reductions in population size faced by many endangered species (Smith & Wayne 1996; Frankham *et al.* 2002; Jehle & Arntzen 2002; Moran 2002) and to the understanding of natural island radiations.

The founder effect was first described by Mayr (1963) as the establishment of a new population by a small number of individuals that derived from a larger source population. The founder population typically carries a small proportion of the genetic variation contained within the original source population. This sampling effect can result in the introduced founder population having distinct genetic and associated phenotypic characteristics which may differ from those contained in the source population. Genetic founder effects have been documented in a number of empirical examples (Baker & Moeed 1987; Clegg *et al.* 2002; Hawley *et al.* 2006). However, some recent studies have failed to detect unequivocal genetic evidence for founder effects in introduced populations (Zenger *et al.* 2003; Durka *et al.* 2005). Several possibilities may explain these inconsistencies. A time lag between introduction and genetic characterization presents an opportunity for new mutations to reduce the genetic founder effect in introduced populations. However, assuming standard mutation rates (Goldstein & Schlötterer 1999) and the short time lags of some studies corresponding to less than 20 generations (Astanei *et al.* 2005; Busch *et al.* 2007), mutation is unlikely to be a significant source of founder population genetic variation. A greater effect on diversity levels is likely through another mechanism that increases within-population genetic variation, that is subsequent introductions of haplotypes not previously introduced to the newly occupied range. Multiple introductions from genetically distinct areas of a source range could feasibly reduce or erase the genetic signature of a founder effect via gene flow (Kolbe *et al.* 2004).

The amount of genetic diversity present in an introduced population is directly related to the amount provided from the source population (Veltman *et al.* 1996). A large founding population is likely to carry a significant amount of genetic diversity, and, as described above, additional variation can be provided by multiple introductions (Astanei *et al.* 2005). A full record of introduction history together with thorough genetic characterization of both native range and introduced populations is rare in empirical studies, but is a necessary component for the full understanding of population genetic processes during introductions. Past analyses have often focused on characterizing the genetic structure of introduced populations, and the comparison of both native range and introduced populations is less common (although see Novak & Mack 1993; Maron *et al.* 2004; Durka *et al.* 2005). Many recent studies have focused on successful introductions that have passed through the initial phases of colonization, establishment and spread, despite the increasing global incidence of human-mediated species introductions that have been observed and documented within decades of initial import (Williams 1969; Shigesada & Kawasaki 1997; Facon *et al.* 2003). Such studies have minimal application to issues of concern in invasion studies, the potential for future establishment and spread of the non-native population, and the relationship between this and the extent of genetic founder effects in introduced populations.

Given the increasingly recognized ecological and economic importance of species introductions, empirical studies are urgently required to elucidate the effects of demographic bottlenecks on the genetic characteristics of early stage introduced populations, and the associated impacts on invasion success (Spencer *et al.* 2000). The discovery of the non-native *Anolis cristatellus* (Sauria: Iguanidae) inadvertantly imported onto the Lesser Antillean island of Dominica within 7 years of the onset of this study, has provided just such an opportunity. Anoles have been widely transported throughout the Caribbean (Losos *et al.* 1993) both by natural dispersal mechanisms such as hurricanes (Calsbeek & Smith 2003; Stenson *et al.* 2004) and through anthropogenic transportation (Campbell 1996). However, the Dominica discovery is rare in that the single point and timing of import is recent and known, its range within the island has been well-documented since an early stage, and the anole niche is filled with a well-studied native competitor, *Anolis oculatus* (Malhotra & Thorpe 1991; Malhotra & Thorpe 1997; Malhotra & Thorpe 2000; Stenson *et al.* 2004).

The nature of *Anolis* community formation in the Caribbean exposes the vulnerability of Lesser Antillean islands to invasion by anoles originating from the Greater Antilles. Lesser Antillean anoles generally experience low levels of competition, because of islands typically containing no more than two species that occupy different microhabitats and niches. Greater Antillean multispecies assemblages with up to 11 species occurring in sympatry on a single island (Losos & Thorpe 2004) have evolved under stronger interspecific interactions to become relatively more competitively robust (Losos & DeQueiroz 1997). Greater Antillean anoles may therefore have a strong competitive advantage when arriving on Lesser Antillean islands, a situation which has only recently been documented (Greene *et al.* 2002; J. Eales, R.S. Thorpe and A. Malhotra, unpublished data), while invasions among islands within the Greater Antilles and among islands within the Lesser Antilles are more common (Fitch *et al.* 1989; Losos *et al.* 1993). The main

import dock on Dominica, as on other Caribbean islands, routinely receives containers from across the Caribbean basin and mainland South America; thus, there are numerous opportunities for cross-island importation of inadvertent stowaways. *Anolis cristatellus* was first identified on Dominica adjacent to the import dock at Deepwater Harbour in 2000, while a survey of the island in 1998 had found no evidence of the alien species (R.S. Thorpe, personal communication). Since importation, the introduced anole has spread approximately 6 km north and 8 km south of the dock along the west coast of the island and has penetrated inland areas within this range up to 200 m altitude. On its native Puerto Rico (Greater Antilles), *A. cristatellus* is the most abundant anole of the 13 species naturally occurring on the island, and occupies a wide range of habitats from sea level to over 700 m altitude (J. Eales, personal observation).

Phylogenetic analysis using mtDNA data has identified a putative source area in central Puerto Rico for Dominican *A. cristatellus* (J. Eales, R.S. Thorpe and A. Malhotra, unpublished data), which we combine here with Puerto Rican nonsource populations and a broad geographical sampling strategy across the introduced range to elucidate spatial population genetic characteristics. This approach extends the scope of this study as a model of species introductions, enabling genetic comparisons between populations of varying introductory history (native source, native nonsource and introduced). Using the unpublished phylogenetic information about the geographical location of putative source populations, in this study we test for a genetic founder effect in Dominican *A. cristatellus* by first comparing levels of genetic diversity among native and introduced populations. Second, we use within-population parameters to test for a genetic founder effect in introduced populations, as compared to native range populations. Finally, we use measures of population genetic structure to determine the partitioning of genetic diversity across native and introduced populations.

Materials and methods

Sample collection

Tail tip biopsies, preserved in 95% ethanol, were obtained from *Anolis cristatellus* lizards in Dominica, Lesser Antilles (introduced range) and Puerto Rico, Greater Antilles (native range). Nine local populations were sampled from Dominica, at four low altitude sites, between 3 m and 41 m above sea level, and four high altitude sites, between 128 m and 176 m above sea level (Fig. 1). Phylogenetic analysis (J. Eales, R.S. Thorpe and A. Malhotra, unpublished data) based on sequencing of a 1433-bp fragment of mitochondrial NADH2 identified three founding haplotypes present in the introduced Dominican population (GenBank Accession numbers to be inserted on acceptance), which were closely related to populations from central Puerto Rico. The present study includes three sample localities from the putative source area in central Puerto Rico and four sample localities in Puerto Rico from outside of the putative source range (Fig. 1). At each locality, 47 tail tips were collected from individual *A. cristatellus*. Population site locations were recorded using a handheld Garmin 60CS GPS.

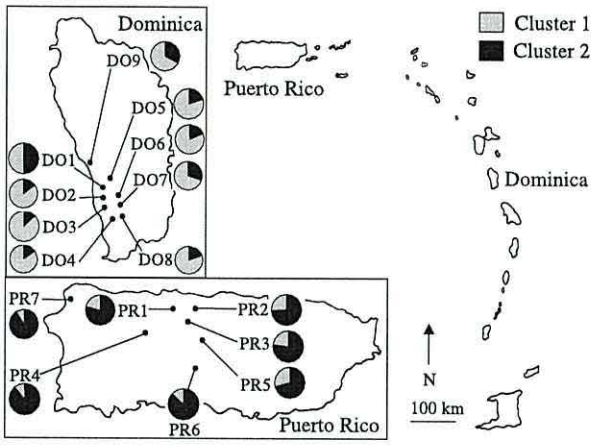

Fig. 1 Location of sampling sites on Puerto Rico and Dominica. Sample localities representing the putative source area on Puerto Rico of the invasive Dominican *Anolis cristatellus* are marked PR1–3. Localities PR5–7 represent nonsource population on Puerto Rico. Pie graphs indicate the proportion of individuals' genomes from each population assigned to each of two clusters identified by STRUCTURE (see text for further details).

DNA extraction and amplification

Genomic DNA was extracted from tail tips using the Chelex extraction method (Walsh *et al.* 1991). Initially, six polymorphic loci were used in this study, developed as molecular markers specifically for *A. cristatellus* (Glor *et al.* 2007). None of the loci used were sex linked. Multiplexed polymerase chain reaction (PCR) amplification was carried out using a reaction volume of 5 μL containing 10 ng of template DNA, 2.5 μL QIAGEN Multiplex PCR Master Mix Q-Solution (QIAGEN), 1 pmol of forward and reverse primers CRIS 22, CRIS 124, CRIS 128, CRIS 136 and CRIS 140 and 1.5 pmol of CRIS 92 (Glor *et al.* 2007). Forward primers were fluorescently labelled. The thermocycling regime consisted of an initial denaturation step at 95 °C for 15 min, followed by 25 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 90 s and extension at 72 °C for 60 s, followed by a final extension phase at 60 °C for 60 s. Labelled fragments were electrophoresed on an ABI 3130 Genetic Analyser (Applied Biosystems) with GeneScan 600 LIZ (Applied Biosystems) as internal size standard. GENEMAPPER version 3.0 (Applied Biosystems) was used to estimate allele sizes using

automatically generated bins, and bin calls were visually confirmed and double checked.

Preliminary data analysis

Only individuals with allele data for all loci were included in the analyses. The data set comprised 527 genotyped *Anolis* from seven native Puerto Rican (three source, four nonsource) and nine Dominican (introduced) populations. Conformance to Hardy–Weinberg equilibrium (HWE) was assessed with exact tests implemented in GENEPOP on the Web (http://wbiomed.curtin.edu.au/genepop/genepop_op6.html) (Raymond & Rousset 1995), which uses a Markov chain method to estimate significance. GENEPOP was also used to test for population-specific departures from linkage disequilibrium between pairs of loci.

Among-population genetic differentiation

Five population-level measures of genetic diversity were calculated in FSTAT (Goudet 2001): allelic richness, the number of alleles per locus weighted by sample size; expected heterozygosity (H_E) as measured by Nei's gene diversity (Nei 1973); observed heterozygosity (H_O) per locus; F_{ST}, the level of genetic differentiation within populations (Weir & Cockerham 1984) and Nei's F_{IS}, the divergence of observed heterozygosity from expected heterozygosity per locus. To test for differences between native range and introduced populations in these parameters, one-sided group comparisons in FSTAT with 1000 permutations were used.

Within-population genetic diversity

Within-population tests for genetic founder effects rely on the expectation that rare alleles are lost rapidly during a population bottleneck and measure this rare allele deficiency relative to other allele parameters to detect a significant effect relative to pre-population bottleneck proportions (Maruyama & Fuerst 1985; Luikart & Cornuet 1998). Two measures were used in this study to detect a within-population genetic signal from a founder event. First, following the expectation that many rare alleles are lost following a population bottleneck, we test for a significant change in allele frequencies at microsatellite loci. Populations at mutation–drift equilibrium are expected to have a large proportion of alleles at low frequency (Luikart *et al.* 1998), typified by an L-shaped allele frequency distribution. Populations that have recently undergone founder events are expected to show a mode-shift distortion in allele frequency distributions to the effect that low frequency alleles become less abundant, resulting in a more even distribution of allele frequencies. BOTTLENECK version 1.2.02 (Cornuet & Luikart 1996) was used to generate the distribution of observed alleles in each population to detect a mode-shift distortion away from the typical L-shaped distribution. Second, because rare alleles are lost more rapidly than overall heterozygosity during a founder event (Nei *et al.* 1975), bottlenecked populations are likely to have higher observed heterozygosities than expected relative to the total number of alleles available (Cornuet & Luikart 1996). Because this effect is most apparent immediately following a population bottleneck, the use of apparent heterozygote excess to detect a population bottleneck may be limited to severe or very recent founder events (Cornuet & Luikart 1996). Heterozygosity excess (relative to the number of alleles detected in each population) was tested in BOTTLENECK under all three proposed models of microsatellite mutation [the infinite allele model (IAM), the stepwise-mutation model (SMM), and the two-phased model (TPM) (70% SMM and 30% variable)], to compare the sensitivity of each. Statistical significances of the results from each model were tested using the Wilcoxon test.

Population genetic structure

To compare genetic structure within and between pairs of populations in the native and introduced range, pairwise F_{ST} (Weir & Cockerham 1984) was calculated between pairs of native range and introduced populations, and between pairs of populations within these population history groupings. Significance of multiple pairwise comparisons was determined using the strict Bonferroni method and the significance level was adjusted to 0.00042 for 120 comparisons. Population F_{ST} and significance value matrices were calculated by FSTAT across 15 000 permutations.

Analyses of molecular variance (AMOVAS) (Excoffier *et al.* 1992) were performed using ARLEQUIN version 3.01 (Excoffier *et al.* 2005), to investigate the distribution of genetic variation among individuals, populations and islands. ARLEQUIN uses both allelic content and frequencies of haplotypes to estimate genetic structure and significance is tested with nonparametric permutations; in this study, 20 000 permutations were used. Separate island AMOVAS were performed for Puerto Rico and Dominica to investigate partitioning of genetic variation within islands. Then, two hierarchical AMOVAS were run to examine the partitioning of genetic variation between islands. First, between Puerto Rican source populations and Dominica, and second, between all Puerto Rican populations and Dominica. The software STRUCTURE version 2.1 (Pritchard *et al.* 2000) uses a Bayesian Markov chain Monte Carlo (MCMC) approach to cluster individuals into population groupings while minimizing Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. The program is most often used to estimate the most likely number of populations based on partitioning of genetic variation, and to determine the average percentage membership of each population to these hypothetical clusters. In this study,

STRUCTURE was primarily used to indicate the strength of genetic partitioning between Puerto Rican and Dominican populations, indicated by the proportion of populations assigned to each of two user-defined clusters (i.e. when $K = 2$). We also used STRUCTURE in the conventional practice to determine the most likely number of clusters the populations may be partitioned into. We ran STRUCTURE for values of K ranging from 1 to 6 with initial burn-in of 10 000 MCMC iterations and a data collection period of 10^6 iterations. Ten independent simulations were run for each value of K and returned consistent results. No prior information was used on the population of origin of each individual. We used the admixture model in which individuals may have mixed ancestry and the correlations model, which takes into account that closely related populations might have correlated allele frequencies. A principal component analysis (PCA) was performed on populations using PCAGEN (www2.unil.ch/popgen/softwares/pcagen.htm), which ordinates populations in multidimensional space using allele frequency data.

Results

Preliminary analysis

All six loci were polymorphic and in linkage equilibrium in each of the populations sampled. Five of the six loci were in HWE, after correcting for multiple comparisons. Fifteen of the 16 sampled populations were heterozygote deficient at locus CRIS 92, thus allele data from this locus were excluded from the subsequent analysis. Departures from HWE in CRIS 92 are unlikely to represent a Wahlund effect (population genetic substructure within predefined populations) because the departures were consistently associated with one locus and not across loci for populations. Because population sampling localities were restricted to 200 m^2 of continuous *Anolis* habitat, and home range size for an individual anole has been estimated up to 34 m^2 (Schoener & Schoener 1982), departures from HWE are unlikely to represent population genetic substructure within the limited sampling areas in this study. A more likely cause of the heterozygote deficiency in CRIS 92 is the presence of null alleles, which result from the preferential amplification of small alleles causing large allele dropout. Other causes of the heterozygote deficit may be close linkage of CRIS 92 to loci under selection or slippage during PCR amplification. Significant departures from HWE were observed in four of the 80 single-locus exact tests for heterozygote deficit in the remaining five loci following sequential Bonferroni correction (Rice 1989). The distribution of these deviations was spread across three loci and four populations and therefore is unlikely to represent any consistent pattern of departure from HWE with either locus or population.

Among-population genetic differentiation

Figure 2 shows pooled allele frequencies per locus for Puerto Rican (PR) nonsource (4), PR source (3) and introduced Dominican (9) populations. At each of the five loci, and overall, introduced populations had fewer alleles (47) than either nonsource (76) or source (68) populations, despite a consistently greater sample size for introduced populations. Three alleles across three different loci were present only in the Dominican populations, in eight, seven and four of the nine introduced populations, respectively. Two alleles were present in PR source and Dominican populations only, and one allele was present in PR nonsource and Dominican populations but not PR source populations. One-sided group comparisons in FSTAT showed significant differences between all PR and Dominican populations in two of the five measures of genetic diversity; allelic richness and H_E (Table 1) with PR populations showing greater

Table 1 A comparison of some population parameters between all Puerto Rican populations of *Anolis cristatellus* and the introduced Dominican population (A) and between three populations in PR (identified as the source of the invading population by mtDNA analysis), and the introduced Dominican population (B). Number of populations is given in column *Npop*. Parameters include allelic richness (A), observed heterozygosity (H_O), expected heterozygosity (H_E), the level of genetic differentiation (F_{ST}), and deviations from HWE (F_{IS}). One-sided probabilities for group comparisons calculated using FSTAT

Island/population history	*Npop*	A	H_O	H_E	F_{IS}	F_{ST}
A						
Puerto Rico	7	9.938	0.798	0.832	0.040	0.043
Dominica	9	8.905	0.774	0.798	0.031	0.033
P value		0.018	0.123	0.017	0.335	0.215
B						
Puerto Rico (source)	3	9.934	0.802	0.837	0.042	0.025
Dominica	9	8.905	0.774	0.798	0.031	0.033
P value		0.070	0.166	0.032	0.372	0.624

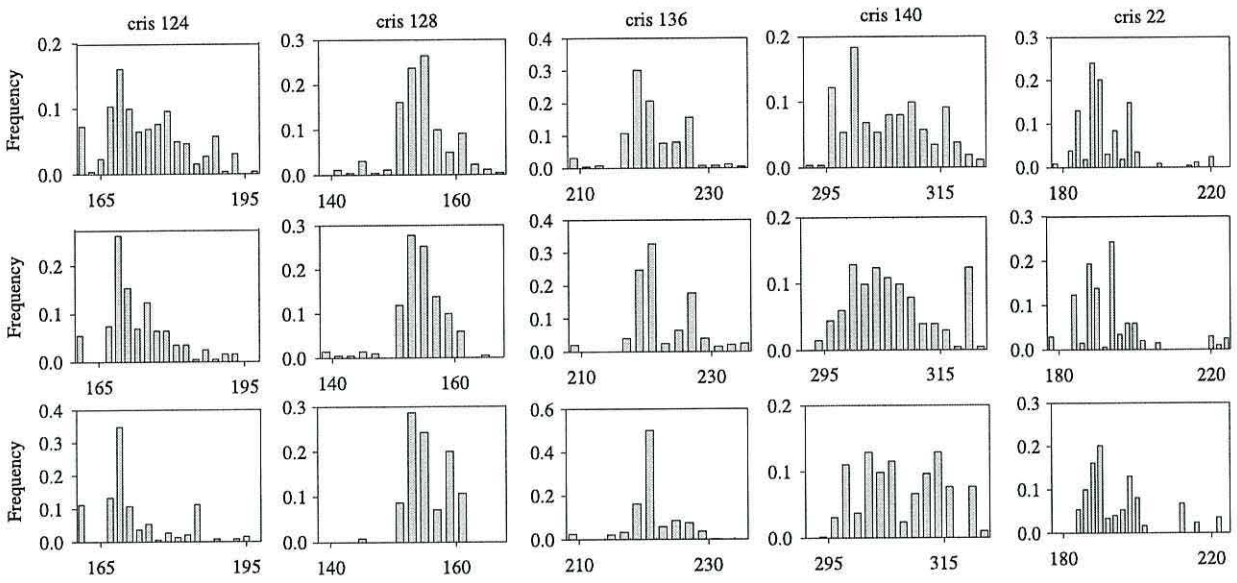

Fig. 2 Pooled allele frequency histograms by locus. First row shows native nonsource Puerto Rican populations (PR4–7), second row shows source Puerto Rican populations (PR1–3), third row shows introduced Dominican populations (DO1–9).

levels of diversity for both measures. Group comparisons between PR source and Dominican populations showed a significant difference in H_E only, with PR source populations showing more expected heterozygosity.

Within-population genetic diversity

There were large differences in results of tests for apparent heterozygosity excess depending on the underlying mutation model employed. Under the IAM, all PR and six of the nine introduced populations showed significant apparent heterozygote excess (Table 2). With an underlying SMM, no population showed significant apparent heterozygosity excess, and under the TPM, one PR source and two introduced populations showed significant apparent heterozygosity excess expected with a population bottleneck: PR3, DO5 and DO6. The IAM is reported to be better able to detect subtle population bottlenecks, although it has also been known to identify them in nonbottlenecked populations (Luikart & Cornuet 1998). Although allele frequency graphs show a reduction in the number of alleles present in introduced populations (Fig. 2), allele frequencies followed an L-shaped distribution, and there was no evidence of a mode shift in allele frequencies towards intermediate values as may be expected in a population that has undergone sequential or prolonged bottlenecks.

Table 2 Tests of within-population heterozygosity excess with three underlying mutational models (IAM, SMM and TPM) performed using BOTTLENECK. Probability values were determined using one-tailed Wilcoxon tests

	Heterozygosity excess *P* values		
Population/history	IAM	SMM	TPM
Puerto Rico (source)			
PR1	0.016	NS	NS
PR2	0.016	NS	NS
PR3	0.016	NS	0.016
Puerto Rico (nonsource)			
PR4	0.031	NS	NS
PR5	0.016	NS	NS
PR6	0.016	NS	NS
PR7	0.016	NS	NS
Dominica (introduced)			
DO1	0.016	NS	0.016
DO2	0.016	NS	0.016
DO3	0.031	NS	NS
DO4	NS	NS	NS
DO5	0.016	NS	NS
DO6	0.016	NS	NS
DO7	NS	NS	NS
DO8	NS	NS	NS
DO9	0.016	NS	NS

Population genetic structure

Significant differences in pairwise F_{ST} values exist between all population pairs (S1) except between eight pairs of introduced populations and one pair of Puerto Rican populations (PR3 and PR5). Values showed typically low amounts of genetic differentiation, ranging from 0.014 to 0.103. Mean pairwise F_{ST} for between-island pairs was

Table 3 Analyses of molecular variance (AMOVAS) showing distribution of genetic variation among islands, populations and individuals for separate island and hierarchical population groups

Range/source of variation	d.f.	Variance components	Percentage of variation	*P* value
Puerto Rico				
Among populations	6	0.095	4.4	0.000
Within populations	457	2.078	95.6	0.000
Dominica				
Among populations	8	0.069	3.3	0.000
Within populations	581	1.995	96.7	0.000
PR (source) and Dominica				
Among groups	1	0.033	1.6	0.007
Among populations within groups	10	0.066	3.1	0.000
Within populations	780	2.020	95.3	0.000
Puerto Rico and Dominica				
Among groups	1	0.045	2.1	0.000
Among populations within groups	14	0.080	3.7	0.000
Within populations	1038	2.030	94.2	0.000

0.058, slightly higher than the mean for Puerto Rican population pairs (0.045) and the mean for Dominican population pairs (0.036). Separate island AMOVAS revealed that limited amounts of geographical structure exist within both Puerto Rico and Dominica, with 4.4% and 3.3% of total genetic variation residing among populations on the islands, respectively, and the remaining 95.6% and 96.7% residing within populations (Table 3). Evidence of high within-population variation is also provided by the results of the hierarchical AMOVA test, which examines the partitioning of genetic variation within and between Puerto Rico and Dominica. This test revealed that the majority of total genetic variation (94.2%) resided within populations, 3.7% resided among populations within islands and 2.1% was due to differences between islands (Table 3). Hierarchical AMOVA indicated that slightly less variation is partitioned between the PR source range (three populations) and Dominica, than between Puerto Rico (seven populations) and Dominica (1.6% and 2.1%, Table 3).

The highest likelihood for the number of genetic clusters identified by STRUCTURE was returned for $K = 2$. Over half the number of individuals within each of the 16 populations were assigned by STRUCTURE to one of the two user-defined clusters representing Puerto Rico and Dominica (Fig. 1). Moreover, more than 70% of individuals' genomes were correctly assigned to the clusters representing the two islands, except for DO1 (51%) and DO9 (67%), demonstrating that despite a low degree of genetic variation between islands (AMOVA), some genetic differentiation exists between Puerto Rico and Dominica. The PCAGEN analysis on allele frequency data provided results concordant with the STRUCTURE analysis. When plotted in two-dimensional space, a clear separation was revealed along the PCA1 axis between Puerto Rican and Dominican populations, except DO1 (Dominica) which groups with Puerto Rican populations,

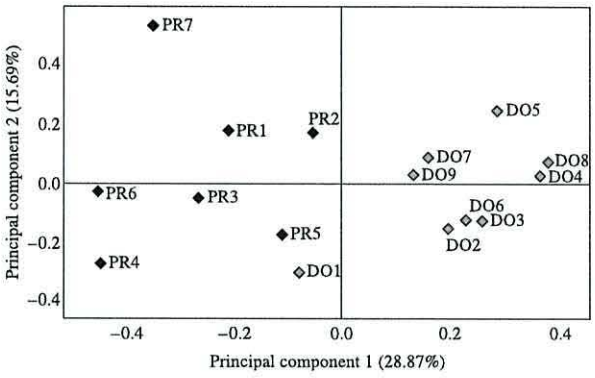

Fig. 3 Principal component analysis scatter diagram based on allele frequency of 16 *Anolis cristatellus* populations from Puerto Rico and Dominica. Putative source Puerto Rican populations (PR1–3); native nonsource Puerto Rican populations (PR4–7); introduced Dominican populations (DO1–9).

Fig. 3. PCA1 represents 28.87% of variation in the data and PCA2 represents 15.69% of variation in the data. These relatively low PCA axis weightings are compatible with a limited degree of between-group or population structuring and the high amounts of within-population genetic variation revealed by the AMOVAS.

Discussion

The general overview from the analyses is that there is limited genetic evidence of a population bottleneck in introduced Dominican populations of *Anolis cristatellus*. This finding raises the question of why a recently introduced, demographically bottlenecked population, should present such a weak genetic founder effect. Losses of genetic variation

during introduction are often modest (Nei *et al.* 1975) and several reasons may exist for this observation. Multiple introductions, even at low levels, are known to have a strong effect on bottleneck signatures and may be a cause of a weak bottleneck signal (Keller *et al.* 2001). However, this is an unlikely cause of the lack of genetic bottleneck on Dominica. Regular interisland shipping throughout the Caribbean has been proceeding for hundreds of years with rapidly increasing frequency, and with only a handful of reported cases of *Anolis* introductions it is unlikely that there have been repeated invasions of a single *Anolis* species on Dominica within 10 years. We suggest that the intricacies of *Anolis* genetics and life history characteristics are likely to play a key role in shaping the weak bottleneck signal presented here.

Most genetic variation lost during population bottlenecks is as a result of genetic drift (Nei *et al.* 1975). When the populations remain at small size for few generations, or rapid population expansion follows the bottleneck, or the founding population is large, genetic drift is negligible. *Anolis cristatellus* on Dominica may have retained variation because insufficient generations were spent at small population size to have lost a significant amount genetic diversity. In the *c.* 7 years between introduction and genetic characterization, *A. cristatellus* has undergone a rapid population expansion, accompanied by a similarly rapid range expansion on Dominica. Although generation time is approximately 12 months, females produce eggs every 2 weeks during the rainy season (J. Eales, personal observation) which may last up to 8 months. In native ranges, anoles occur at high abundances of up to 0.97/m^2 (Schoener & Schoener 1980) which are consistent with those observed across much of the *A. cristatellus* Dominican range. As a result, it is likely that the number of generations with a small population size has been few, and consequently, that genetic drift has been minimal for this introduction. Furthermore, although the size of the initial founding population has been cited an important factor contributing to the genetic bottleneck (Veltman *et al.* 1996), it is the amount of genetic variation contained within that population that is most significant. AMOVAS showed that the majority of genetic variation exists within *A. cristatellus* populations, rather than between populations or groups of populations, indicating limited genetic structuring (probably because of high gene flow) even in Puerto Rican populations. Thus, a small founding population sourced from within a native Puerto Rican population will contain a relatively high degree of genetic diversity, facilitating high genetic diversity in Dominican *A. cristatellus*.

One particular aspect of *Anolis* reproductive ecology presents an important mechanism by which genetic diversity can be maintained during a founder event, accounting for the weak bottleneck signal described in this study. The phenomenon of sperm storage has been described in many squamates (Cuellar 1966; Pearse & Avise 2001; Sever & Hamlett 2002) and female *Anolis* have specialized vaginal sperm storage tubes specifically for this purpose (Fox 1963). Since female *Anolis* ubiquitously mate with multiple males (Tokarz 1998), sperm storage introduces the potential for multiple paternity of offspring. A study by M.A. Johnson, R. Kirby, C.C. Fresquez, S. Wang (submitted) found that 52% of *A. cristatellus* females that had produced two or more progeny had mated with multiple males, and for *Anolis sagrei*, the figure was 81% (Calsbeek *et al.* 2007). The data for the length of time that female *Anolis* are able to store sperm and produce viable offspring ranges from 107 days (*A. sagrei*, Calsbeek *et al.* 2007) to 10 months (*Anolis carolinensis*, Passek 2002). These reproductive characteristics have significant implications for the potential genetic contribution by female *Anolis* that have been sexually active in the source range before introduction. Female *A. cristatellus* can produce up to six viable offspring under isolated laboratory conditions (J. Eales, personal observation), thus each sexually active female can potentially introduce the genotypes of up to six individuals of different paternity to introduced populations. Based on the presence of at least seven sexually mature females in the initially transported founding population (deduced from prior mtDNA analysis, J. Eales, R.S. Thorpe and A. Malhotra, unpublished data), multiple paternity could be responsible for the introduction of up to 42 genotypes in Dominica. These figures are upper estimates, in light of empirical data from *Anolis* females (Calsbeek *et al.* 2007; M.A. Johnson, R. Kirby, C.C. Fresquez, S. Wang, submitted). However, the founding population was not necessarily restricted to seven females, and females with identical mtDNA haplotypes may also have been present in the group transported to Dominica. Together, these factors may result in a much higher initial effective population size for *A. cristatellus* than the actual number transported. The capacity for the introduction of varied paternal genotypes by sexually active females in the initially transported population may be a substantial contributor to the genetic diversity presented in this study. These aspects of squamate reproductive ecology may represent an important mechanism by which introduced populations of this order might minimize the extent of genetic founder effects resulting from demographic bottlenecks.

The genetic signal from a demographic bottleneck is most apparent in the loss of rare alleles (Maruyama & Fuerst 1985), followed by a reduction in the mean number of alleles (Luikart *et al.* 1998). Although there was no significant alteration of the L-shaped allele frequency distribution, fewer alleles (total number of alleles and number of alleles per locus) were present in introduced as compared to native and source populations, even though sample sizes were larger for Dominican populations. This observation is concordant with lower allelic richness and H_E in Dominican

than Puerto Rican populations, and together provide evidence for loss of variation in some genetic diversity measures. However, there was no conclusive and consistent evidence of loss of genetic diversity in introduced populations from pairwise F_{ST} comparisons or group comparisons of H_O, F_{ST}, and F_{IS}.

The use of heterozygote excess to detect a population bottleneck may be limited to severe or very recent bottlenecks (Cornuet & Luikart 1996). Tests for heterozygote excess (relative to the number of alleles in a population) showed no evidence of a founder effect in introduced Dominican populations, and was inconsistent across mutational models, an observation which has also been reported in recent studies (Hufbauer *et al.* 2004; Hawley *et al.* 2006; Aketarawong *et al.* 2007). The Wilcoxon test for the significance of heterozygosity excess was used because it has been cited as the most robust of the significance tests implemented by the program. However, it has most power when 10–25 loci are used (Piry *et al.* 1999), and therefore may be of limited value in this study (five loci). Because microsatellites rarely follow a strict mutational model, programs that rely on mutational models may be of limited value.

Several lines of evidence raise the question as to whether the founding population on Dominica is fully represented by the three sample sites from the putative source area in central Puerto Rico. First, an allele which is present in both Dominican and PR nonsource populations was identified. Two explanations may account for this finding. Either the true PR source comprises a larger geographical area than the putative source, or the allele is present in, but was not sampled from the putative source populations. Second, three private alleles were identified on Dominica. High fecundity of anoles (each female produces an egg approximately every 14 days during the rainy season) has led to high abundances on Dominica (numbering into the hundreds of thousands) and given the current estimates of microsatellite mutation rates (in the region of 5×10^{-4}, Goldstein & Schlötterer 1999) it is possible that new alleles may have been created in the *c.* 7 years between introduction and genetic characterization. However, because of the wide distribution of the three private Dominican alleles in populations across the Dominican range, they are likely derived from the PR source, rather than arising from new mutations occurring in the short time before dispersal of the founder population. Together with results from AMOVAs and Pairwise F_{ST} estimates, these observations provide strong evidence that significant amounts of genetic variation exist within Puerto Rican populations, such that despite substantial sample sizes, not all alleles in a population can be represented by the samples of populations used in this study. A high degree of within-population variation was also inferred by mtDNA analyses: individuals taken from the same population of *A. cristatellus* frequently had different haplotypes, a common observation in other anole phylogenetic studies (Malhotra & Thorpe 2000; Glor *et al.* 2001; Kolbe *et al.* 2004). Consequently, there is no unequivocal evidence to suggest that the three sample sites do not adequately represent the geographical source of the population on Dominica. It is more likely that there is simply a very high degree of within-population variation. Even so, the categorization of source and native Puerto Rican populations should be treated with caution when interpreting the results.

Our results provide valuable insights into the relationship between genetic diversity within source and introduced populations and the success of introductions in the early stages of invasions, information which may be applied to other recent introductions. We have provided a clear representation of the degree of genetic variation present in a recent (less than 10 generation) species introduction at the fine-scale microsatellite level. Evidence presented here suggests that high population genetic diversity present in a species' native range may be transferred to introduced populations with minimal genetic founder effects and result in a highly successful invasion, despite the founding population originating from a limited geographical area. Previous studies have shown that a large amount of genetic variation in introduced *Anolis* populations results from admixture from different geographical areas of the native range (Kolbe *et al.* 2004). Conversely, here we demonstrate that admixture is not a necessary requirement for observed high genetic diversities in introduced populations. A high degree of gene flow within source areas can help ameliorate founder effects when founding populations originate from a restricted area. We suggest sperm storage as a major contributor to the observed genetic diversity in introduced Dominican *A. cristatellus* populations, and propose this as an important mechanism in minimizing genetic diversity losses during demographic founder events in *Anolis* and other squamates.

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This work was carried out as part of Jacqualyn Eales' PhD thesis (supervised by RST and AM) on the population genetics and evolutionary ecology of *Anolis* introductions in the Lesser Antilles. Royer S. Thorpe and Anita Malhotra have investigated natural selection, population genetics, molecular phylogeography and speciation in island lizards as part of their broader research activities of the Molecular Ecology and Evolution of Reptiles Unit in Bangor. The other focus of interest in this unit is the evolution of venomous snakes and their venom.

Erratum.

Appendix V

The following sentence in the Materials and Methods section on page 3 of Eales *et al.* 2008 should have detailed and included seven GenBank Accession numbers to read:

Phylogenetic analysis (J. Eales, R.S. Thorpe and A. Malhotra, unpublished data) based on sequencing of a 1433-bp fragment of mitochondrial NADH2 identified seven founding haplotypes present in the introduced Dominican population (GenBank Accession numbers EU599076-82), which were closely related to populations from central Puerto Rico.