

Genomics and adaptive radiation on remote islands

The islands' story

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"...the very close relation of the distinct species which inhabit the islets of the same archipelago, — and especially the striking relation of the inhabitants of each whole archipelago or island to those of the nearest mainland, — are, I think, utterly inexplicable on the ordinary view of the independent creation of each species, but are explicable on the view of colonisation from the nearest and readiest source, together with the subsequent modification and better adaptation of the colonists to their new homes."

So wrote Charles Darwin in *On the Origin of Species* (1859)¹. The "very close relation of the *distinct* species" that he observed on numerous occasions, most famously the Galapagos finches, is of course the product of adaptive radiation, a term attributed to the American palaeontologist H.F. Osborn². An adaptive radiation can be defined as the rapid diversification of a lineage into multiple ecologically different species. In Darwin's spirited observation, he encapsulated the four criteria that define an adaptive radiation:

- i. that the species share a common ancestry
- ii. that there is a correlation between species' phenotype and their environment
- iii. that the species' phenotype includes traits that are useful (adaptive) in their environment
- iv. that speciation has occurred rapidly

It is likely that episodes of adaptive radiation have generated much of the diversity of life; however, most of the best-known examples come from remote islands, archipelagos or similar settings. From Darwin and Wallace on, the concepts and theories of adaptive radiation and much of evolutionary biology have been developed through studies on islands. Indeed, the parlance of population genetics is pregnant with the reasoning invoked by islands – we talk of 'island models' and sample organisms from 'populations' which are then often treated as 'islands' and their degree of genetic divergence tested. Both real and imagined islands are amenable to study because of their discreteness. In this short article, we briefly define what we mean by remote islands, why they promote adaptive radiation and illustrate how molecular genetics and genomics are transforming our understanding of the adaptive process using examples from our own work on spiders and other key examples.

inhospitable matrix³. Typically, we imagine an oceanic island surrounded by a matrix of seawater; however, from the perspective of particular organism, an isolated rock, tree, mountaintop ('sky-islands'), cave system, river or lake may represent an island depending on its degree of isolation; which in turn depends upon the ability of the organism to disperse through or across that matrix. Although isolation is fundamental, the history of an island – how and when it came to be – critically determines the composition of the biota and the nature of the evolutionary and ecological processes that occur (for a detailed review see reference 3).

Truly remote islands, for example volcanic oceanic islands, are typically formed *de novo* and are initially devoid of life (in contrast with fragment islands that are formed when an area of habitat becomes separated from a previously contiguous habitat and consequently have their ecological niche space already largely filled at the time of isolation). On these Darwinian islands (so called because Darwin promoted this, at that time controversial, mechanism¹), the number of species is initially zero and increases over time. On highly isolated islands, the number of colonizing species that can reach the island is extremely small and their rate of immigration is much lower than the rate at which new species can form. Multiple neo-endemics may form through adaptive radiation. The outer limit for the dispersal ability of an organism is referred to as its radiation zone and one result of the differential abilities of species to disperse is that the fauna and flora of islands tends to be disharmonious – statistically non-representative of the mainland or source. This disharmony increases with distance from the source, as fewer and fewer lineages are able to colonize – a process called attenuation. Consequently, many higher taxa tend to be poorly represented on remote islands. The Hawaiian archipelago is extremely isolated (4000 km from the North America and 3200 km from the nearest island group) and exhibits extreme disharmony. Only 50% of insect orders and 15% of the known families are

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Remote islands as Darwinian islands

Broadly, an island can be defined as any discrete habitat that is isolated from other areas of similar habitat by an

represented⁴, and most species are endemic and are the product of numerous adaptive radiations.

Remote Darwinian islands facilitate adaptive radiation because of attenuation and because, at least early in the history of the island, ecological niche space is wide open to those lineages with high dispersal abilities that manage to colonize. In contrast, the members of an island adaptive radiation typically have greatly reduced dispersal abilities, and this loss may be an important factor in subsequent population fragmentation and diversification. New colonists frequently expand their ecological range ('ecological release'). This may occur because of some 'key innovation' that the colonizer has that allows it to exploit the novel environment, or because of inherent ecological or behavioural plasticity that allows a species to occupy new environments in the face of relaxed competition. Selection may then act on the initial minor behavioural or morphological differences to drive differentiation⁵. In a similar way, when populations are small, in lineages where sexual selection plays a major role, female discrimination may be relaxed, leading to simpler and more variable sexual behaviour. Subsequent isolation may then lead to differentiation through sexual selection and shifts in the distribution of mating preferences. Such a mechanism probably explains the spectacular radiation of the Hawaiian *Drosophila*⁶ and of the Hawaiian *Laupala* crickets⁷. The latter has the highest speciation rate recorded in arthropods and closely related species differ only in the male courtship song.

Nonetheless, the exact mechanisms of how widespread and dispersive species give rise to differentiating and then fragmented and finally specialized endemic species, and if and why certain lineages are more disposed to adaptive radiation than others, are poorly understood.

Molecular genetics, genomics and phylogeography

Molecular genetics and genomics are transforming our understanding of both the history of adaptive radiations and the basis of adaptive change. Through molecular phylogenetics, typically based on sequence data from mitochondrial or chloroplast DNA and/or nuclear genes, we are able to trace in detail the relationships among the members of an adaptive radiation, determine their likely source, their patterns and their rates of colonization, radiation and population expansion throughout an archipelago. The availability of numerous anonymous markers such as AFLP (amplified fragment length polymorphism) is also making an impact in this area by enabling us to infer the degree and role of hybridization, admixture and direction of gene flow in adaptive radiation; when gene-based markers may give conflicting results. An example of the power



Hawaiian islands. The archipelago of Hawaii, about three thousand kilometres west of Los Angeles, California, is made up of seven islands. They are, from left to right, Niihau (obscured by clouds), Kauai, Oahu, Molokai, Lanai, Kahoolawe, Maui, and Hawaii (the 'Big Island'). Image from the Moderate Resolution Imaging Spectroradiometer (MODIS) on NASA's Terra satellite (NASA)

of phylogenetic inference in elucidating an adaptive radiation is the 'spiny leg' clade of Hawaiian *Tetragnatha* spiders⁸. The Hawaiian islands have been of particular interest in the study of adaptive radiation, not only because of their isolation, topographical diversity and consequent high degree of endemism and adaptive radiation, but also because they, like many other oceanic island systems, are a volcanic 'hotspot' archipelago, with a chronological sequence of islands (ranging from Kauai at 5.1 million years old to Hawaii at less than 1 million years and still forming). Such islands are therefore natural laboratories for evolutionary studies as replicate communities of closely related individuals may be found on multiple islands and within islands (because of the complex volcanic topography). The 16 species of spiny leg *Tetragnatha* exhibit a phylogeography typical of many other Hawaiian taxa – a pattern of colonization and subsequent diversification along the island chain from the oldest island to the youngest. What is remarkable about this lineage is that different forest communities each contain up to four specialized ecomorphs of different colour and size and that each can be mapped to one of four ecological roles: a green, active, leaf-dwelling form feeding on flying insects; a maroon moss-dwelling form; a large brown, slow-moving and bark-dwelling form that mostly feed on Lepidoptera larvae; and a small brown, active, twig-dwelling form that feeds on very small insects (Figure 1). In no community is a particular ecomorph represented by more than one species. Species' phylogenies tend to cluster by island rather than ecomorph, indicating that the same non-competing ecological forms have evolved repeatedly to create similar community assemblies following colonization. Similar extensive within-locality proliferation and repeated evolution of equivalent ecomorphs in different localities has also occurred in the spectacular radiations of cichlid fish in the African Great Lakes⁹, sticklebacks in Canadian glacial lakes¹⁰, and *Anolis* lizards in the Caribbean¹¹. It seems that adaptive radiation is able to overcome the historical vagaries that suggest that repeated evolutionary trajectories, even from the same initial conditions, would be unlikely to reach the same end product.

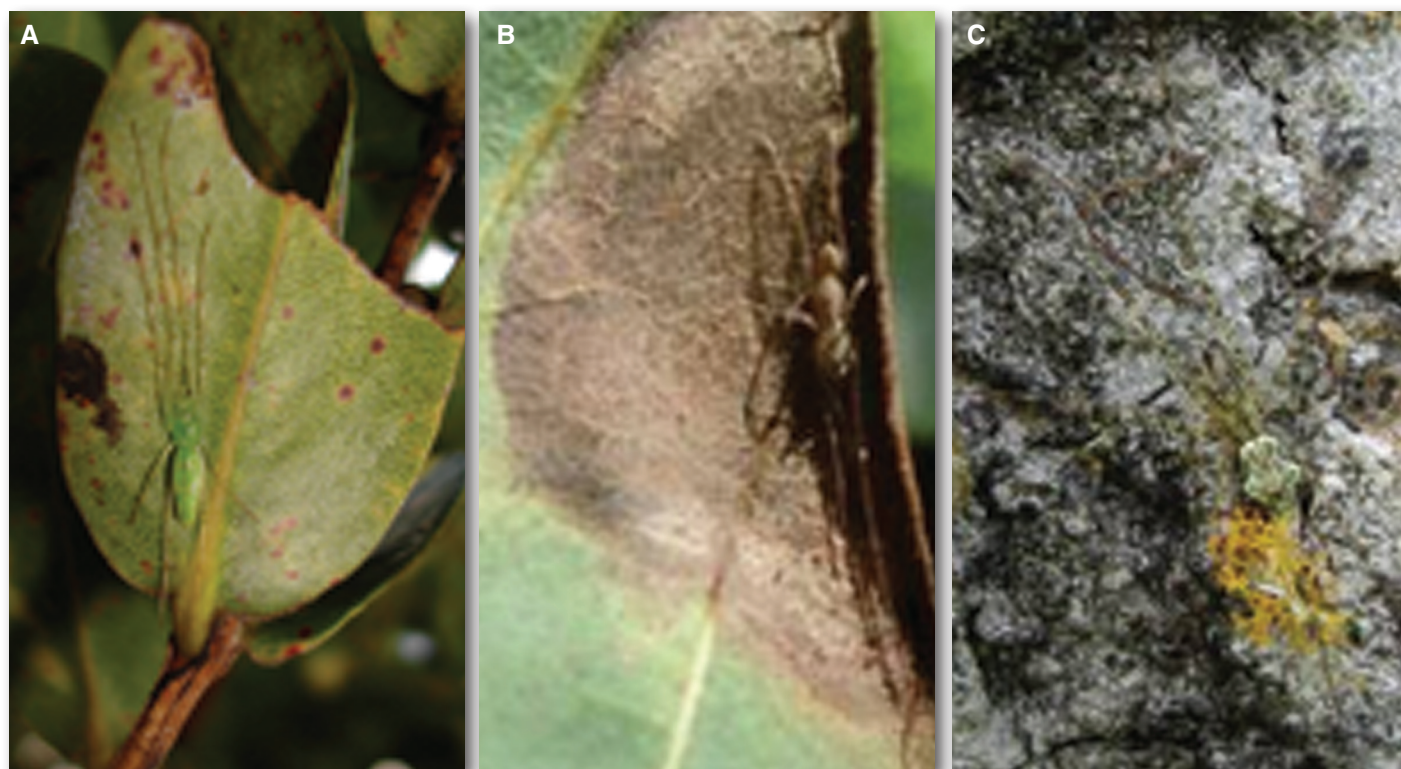


Figure 1. Members of the Hawaiian spiny leg clade of *Tetragnatha* spiders illustrating high-fidelity background choice for maximum crypsis given their ecotype. (A) *T. kauaiensis* (green ecomorph, Kaua'i); (B and C) *T. quasimodo* (large brown ecomorph, Hawai'i). (Photos: R. Carter)

Genomics and the gift of parallel adaptive radiations

Repeated instances of adaptive radiations leading to parallel adaptive phenotypes, combined with molecular genetic and genomic approaches, have allowed tremendous progress in elucidating the molecular basis of adaptive change in several taxa such as *Anolis* lizards on Caribbean islands¹², cichlid fish in the African Great Lakes¹³ and, perhaps most spectacularly, the three-spined stickleback *Gasterosteus aculeatus*. This typically marine fish is unusual in being both a model vertebrate organism and one that has undergone a recent and extensive adaptive radiation as marine populations became isolated in numerous lake and stream systems across the Palearctic following the last glaciation. These lakes are effectively remote 'islands'. A full genomic toolkit is available for this species, including laboratory lines, cDNA, EST (expressed sequence tag) and BAC (bacterial artificial chromosome) libraries, physical maps and now a genome sequence. Among numerous phenotypic changes that have occurred, the most notable is the consistent reduction in lateral armour plates observed in all freshwater populations. Hybrids between freshwater and marine populations indicated that armour plating is inherited in a simple dominant fashion, and complementation tests between Japanese and Canadian freshwater populations were negative, indicating that the same locus was responsible for plate loss¹⁰. This locus has subsequently been identified as *Ectodysplasin* (*Eda*) through genome-wide linkage mapping using microsatellite and AFLP markers, with subsequent positional refinement through linkage disequilibrium (association) mapping in natural populations¹⁴. In a similar manner, candidate genes for the orange blotch locus, an important player in cichlid sexual selection and diversification, have also been mapped¹³. The stickleback identification of the *Eda* locus has also been replicated through a hitchhiking analysis of microsatellite markers in the region¹⁵. This approach, which starts with a genome-wide scan of mic-

rosatellite or AFLP markers in natural populations, aims to identify markers that are in linkage disequilibrium ('hitchhiking') with loci experiencing recent natural selection. Loci, and neighbouring markers, that have experienced a positive (divergent) selective sweep display higher than expected levels of divergence (F_{ST}) than the background genome-wide estimate. Recent statistical advances in the treatment of dominant markers such as AFLP and the application of genome-wide scans for selection, linkage-disequilibrium mapping and admixture mapping¹⁶ make it possible to identify the molecular signatures of adaptation in non-model species. New low-volume sequencing technologies will permit the genes behind many of these loci to be identified and the role of selection on their evolution at the sequence level to be verified using classical tests such as the McDonald-Kreitman test and its derivatives (see reference 17 for a review).

Genomics has also been successful in providing molecular evidence for the role of sexual selection in adaptive change. Jagadeeshan and Singh¹⁸ demonstrated, by comparing transcript expression levels between closely related *Drosophila melanogaster* and *Drosophila simulans* flies, that reproductive tissues contained more REGs (rapidly evolving genes) than non-reproductive (head) tissues. They demonstrated a higher proportion of REGs

in testes than in ovaries, indicating higher selective pressure and more rapid evolution in the male reproductive system. Furthermore, these genes showed strong signatures of positive selection at the sequence level. In a similar vein, parallel evolutionary changes in expression levels of gene transcripts have been observed in farmed salmon from Canada and Norway when compared with wild populations¹⁹.

Parallel adaptation without radiation

It can also be informative to examine taxa which exhibit diversity but without adaptive radiation. Adaptive radiation implies divergent (positive) natural or sexual selection; however, adaptation may also involve non-diverging balancing selection – maintaining polymorphic variation within populations and limiting between population diversification of the traits concerned. Little attention has been given to this area, but remote island archipelagos again provide a unique opportunity to understand the molecular evolutionary basis of balanced polymorphisms in independent and otherwise divergent replicate populations. An excellent example of this is the Hawaiian happy face spider *Theridion grallator* (Figure 2). This species exhibits a remarkable colour polymorphism, with more than 20 intricate yellow, red and black dorso-abdominal patterns described. The colour morphs are allelic and essentially exhibit a simple dominant pattern of inheritance at a single locus relative to the recessive ‘plain yellow’ morph. The spider is found in isolated and otherwise genetically divergent populations on each of the main Hawaiian Islands and, remarkably, the same set of colour morphs has re-evolved and is maintained, apparently *de novo*, in parallel, in each population. It therefore seems likely that the polymorphism is controlled by the same locus (though not necessarily the same mutations) in each population²⁰. Similar colour polymorphisms (with patterns convergent to many of those found in *Theridion grallator*) are found in other unrelated members of the Theridiidae spider family, on both islands and continental land, suggesting that they are the product of the same canalized ontogenetic process. The repeated generation of the polymorphism in *T. grallator* renders it a model for the similarly repeated evolution of this polymorphism across the family.

All these species share a common ecology; they have largely abandoned web-building and use the underside of leaves as both capture surfaces and retreats, presumably exposing themselves to predation relative to their cobweb-spinning relatives. The Hawaiian spiny leg *Tetragnatha* spiders also retreat to a web-less substrate for shelter. Why then have these species radiated into specific ecomorphs while *T. grallator* maintains a balanced colour polymorphism? Both appear to be predator-avoidance strategies based at least part on crypsis. Perhaps one answer is

related to the observation that spiny leg *Tetragnatha* spiders return, after a night's hunting, to their ecomorph's appropriate background vegetation with almost 100% fidelity. *Tetragnatha* have poor vision and so may use chemical cues from the vegetation, in a similar manner to many herbivorous insects and their predators that are attracted to volatile chemicals (semiochemicals) released, for instance, when a leaf is damaged. In *Tetragnatha* then, it seems likely that some locus defining chemical preferences must be linked directly to – and evolve in concert with – phenotypic changes in body colour, facilitating crypsis. Such co-selection on other adaptive traits such as salt-tolerance, parasite-resistance and behaviour may also have occurred in freshwater populations of sticklebacks in genes closely linked to *Eda*. This may not be the case in *T. grallator*, which instead has evolved a classic colour polymorphism that probably operates in a frequency-dependent manner similar to that of many land snails. Although the molecular basis of this colour polymorphism is not yet known, we are exploring this system with the same array of genomic tools (i.e. AFLP-based genome scans for selection, linkage disequilibrium and genetic mapping) that are proving so fruitful in dissecting the basis of positive adaptive evolution in island radiations.

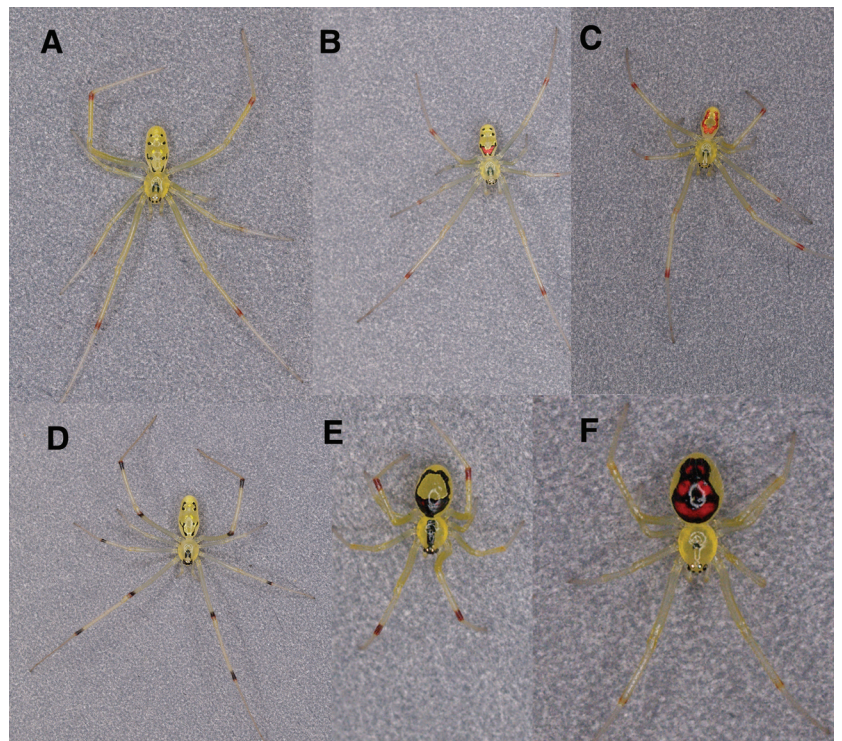


Figure 2. The Hawaiian happy face spider *Theridion grallator* exhibits an exuberant adaptive colour polymorphism that is found in all populations. (A) Yellow morph (Hawai'i); (B) Red front morph (Hawai'i); (C) Red lines morph (Hawai'i); (D) Yellow morph (Hawai'i); (E) Black ring morph (O'ahu); (F) Red-black ring morph (O'ahu). (Photos: P. Croucher)

Cataloguing a remote island biota – the Moorea Barcode project

The 134 km² island of Moorea is located in French Polynesia in the south Pacific, approximately 20 km west of Tahiti. This remote island is the focus of an unprecedented and ambitious project between the University of California's Richard B. Gump South Pacific Research Station and France's Centre de Recherches Insulaires et Observatoire de l'Environnement (<http://moorea.berkeley.edu/biocode/>). The project, funded by the Gordon and Betty Moore foundation, aims to catalogue and DNA barcode all 5000+ species of plant, animal and fungi both on the island and in the surrounding reefs, including gut contents. DNA barcoding implies obtaining the unique DNA sequence from a fragment of the mitochondrial cytochrome oxidase I gene (*COI*) (*ITS4* for fungi) that characterizes each species, just as a barcode uniquely identifies a grocery item. In this way, the project aims to elucidate how all the organisms fit together within the ecosystem, to reconstruct the entire food web and understand the phylogenetic relationships between the taxa in each class. This project will generate volumes of data about the adaptive radiations that have occurred on Moorea and the origins of the lineages concerned. Moreover, it will generate a model system for understanding how island biotas respond to disturbance from invasive species and climate change. Never before have molecular techniques been applied to an ecosystem at this level.

The Moorea Biocode Project is a prime example of why this is an auspicious time for ecologists and evolutionary biologists. Just as genomics and molecular biology have revolutionized other areas of biology such as clinical science, physiology, development and systematics, these tools are now making it possible to understand the processes of speciation, adaptation and community

assembly at the molecular level. Perhaps nowhere will this impact be greater than on island systems, which have provided the fundament and inspiration upon which much of evolutionary and population genetic theory is based. After 150 years of the *Origin of Species*, remote islands, the systems that inspired Charles Darwin so profoundly, remain at the forefront and continue to surprise and drive evolutionary biology. ■



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