

Project Report

Ecological Divergence in a Tropical Tree: *Protium subserratum* (Burseraceae)

Neotropical forests have the highest tree diversity on earth, with an estimated 22,000 species¹ a stark contrast to the 1,166 tree species described for North America, Europe and Asia combined². The process of new species formation has been intriguing biologists ever since Darwin and Wallace first posited the theory of natural selection; however the majority of evolutionary studies today primarily focus on temperate flora and faunal systems. The Amazon rainforest, the most bio-diverse place on earth, and subsequently where the evolution of new species has been hypothesized to occur at a comparatively higher rate and frequency has been largely ignored.

When compared to the mountains, deserts, canyons and coasts of more temperate regions the lowland Amazon basin is often mistakenly thought of as a contiguous stretch of singular rainforest habitat while in fact it encompasses a vast amount of environmental heterogeneity. The lowland rainforest of Northwestern Peru is particularly noted for its mosaic of different forest types, each housing a different suite of unique animal and plant inhabitants. Particularly notable are the white sand forests which are found on ancient quartz sands dating prior to the Andean uplift and which are patchily distributed as habitat 'islands' surrounded by more common clay and terrace soils³. Because many endemic tree species found on these white sand soil types are often very closely related to tree species growing nearby in clay soil forests they provide the perfect opportunity to study how ecological adaptation and natural selection can drive the formation and maintenance of new species.

The *Protium subserratum* species complex (Burseraceae) provides a rare opportunity to study the isolating mechanisms that contribute to ecological divergence as it is occurring. The genus *Protium* (Burseraceae) is a diverse clade of ca. 140 tree species, 100 of which are found in the rainforests of the lowland Amazon Basin⁴. While the majority of *Protium* species are soil specialists, *P. subserratum* represents one of the few soil generalists in the genus, with genetically and morphologically differentiated subspecies found on the more fertile clay soils and the nutrient-poor white sand soils⁵. The tree species is widespread and has morphologically differentiated subspecies found on patches of clay and white sand soil habitat found directly adjacent to each other. Additionally, while population genetic analysis suggests the presence of low gene flow across habitat boundaries populations of both morphotypes are clearly maintaining their genetic and morphological integrity, suggesting that strong barriers to reproduction exist.

The research described here aims to fill a critical missing link in the evolution literature by examining the strength and role of isolating barriers that occur before and after fertilization in two subspecies of the tropical tree, *Protium subserratum*.

Barriers to gene flow that occur prior to fertilization are often dictated by the presence or absence of sex. If two groups of organisms do not to exchange gametes, whether they are capable of producing offspring or not, they are considered reproductively isolated. When considering plant reproduction things are slightly more complicated. Because many plants rely on animals for reproduction, the distance, direction and degree of (pollen) dispersal are directly influenced by the movement of animal pollinators^{6,7}. As a result, animals are often ultimately responsible for determining the spatial pattern of gene movement within and between plant populations. Pollinator specialization can result in complete reproductive isolation if a change in floral type (i.e. color, size, or shape) results in a switch to a new type of pollinator⁸.

Alternatively, pollinators may be indiscriminate generalists, leading to high levels of gene flow between populations.

P. subserratum individuals produce either male or female flowers and both subspecies overlap in flowering time. Flowers are small, white, nectar producing, and sweet smelling, indicating that pollinators may be floral generalists, such as bees which will visit any flower with a nectar or pollen reward. If pollinators are generalist visitors then environmental barriers to reproduction prior to fertilization may be weak and barriers to reproduction may be stronger after fertilization has occurred.

Reproductive barriers that occur after fertilization can be *genetic*--where maladapted gene complexes result in hybrid mortality--or *environmental*--where hybrids are poorly adapted for survival in either parental habitat⁹. Population genetic analysis suggests the low presence of adult hybrid individuals in clay soil habitats, indicating that genetic barriers to gene flow may not be present. If this is the case then environmental barriers occurring after fertilization could be responsible for the isolation of white sand and clay soil subspecies. If this is the case we would expect that seed set from 'hybrid' cross-pollinations would be just as successful as parental crosses however after germination hybrid seedlings would experience lower fitness in either soil type compared to the respective parental type seedlings.

We tested the predictions that different insects are not specializing on different subspecies of *P. subserratum* and that hybrids are rarely found in nature because they are not adapted to survive in either soil type using insect observations and *in-situ* hand pollination experiments.

Insect visitors to both subspecies were observed while both subspecies were flowering. Insect observation was conducted using digital video recordings which were mounted in flowering trees of each sex in both white sand and clay habitat types. Eleven hours of high definition recording was captured and is currently being reviewed to identify floral visitor as well as to determine the duration and frequency of floral visitation in each habitat type. Behavior, such as pollen and/or nectar collection and grooming at the flower, will be assessed to identify effective pollinators.

In order to test for reproductive isolation after fertilization hand cross experiments were conducted. Female inflorescences were bagged while flowers are still in bud to ensure no prior pollination. Hand pollinations were made by gently tapping pollen bearing anthers of male flowers on the receptive stigma of female flowers. After pollination, flowers were re-bagged for two weeks and then checked for fruit formation.

A total of 178 cross pollinations were made. One hundred forty hybrid pollinations were made between the white sand and clay subspecies and 38 parental type cross pollinations were made between males and females of the same subspecies. Two weeks after pollinations hybrid crosses demonstrated fruit set in 16.4% of crosses while parental crosses showed successful fruit set in 39% of crosses. This result demonstrates that fertilization between subspecies is possible however whether the lower seed set between hybrid crosses is due to genetic mechanisms resulting in low fertilization rates or the result of high mortality or fruit abortion post fertilization is equivocal.

Tropical rainforests are frequently noted for their high levels of plant biodiversity, yet significantly fewer studies of speciation in the tropics exist compared to temperate zone systems. These preliminary experiments are a first step in the process of thoroughly addressing tropical tree diversification and are among the first to use a tree system to explicitly examine the role of natural selection in the evolution of reproductively isolating mechanisms.

- ¹ Latham RE and RE Ricklefs. 1993. Continental comparisons of temperate-zone tree species diversity. In: Ricklefs RE and D Schluter. (eds) *Species diversity in ecological communities*. University of Chicago Press, Chicago, pp 294-314.
- ² Fine PVA and RH Ree. 2006. Evidence for a time-integrated species-area effect on the latitudinal gradient in tree diversity. *American Naturalist* 168: 786-804.
- ³ Gentry AH 1988. Patterns of plant community diversity and floristic composition on environmental and geographical gradients. *Annals of the Missouri Botanical Garden*. 75: 1-34.
- ⁴ Daly, DC. 1987. A taxonomic revision of *Protium* (Burseraceae) in Eastern Amazonia and the Guianas. Ph.D. diss., City University of New York, New York.
- ⁵ Daly DC, and PVA Fine. 2011. A new Amazonian section of *Protium* (Burseraceae) including both edaphic specialist and generalist taxa. *Studies in Neotropical Burseraceae XVI. Systematic Botany*. 36: 939-949.
- ⁶ Dick CW, G Etchelecu, and F Austerlitz. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12: 753-764.
- ⁷ Garcia C, P Jordano, and JA Godoy. 2007. Contemporary pollen and seed dispersal in a *Prunus mahaleb* population: patterns in distance and direction. *Molecular Ecology* 16: 1947-1955.
- ⁸ Campbell DR. 2008. Pollinator shifts and the origin and loss of plant species. *Annals of the Missouri Botanical Garden* 94: 264-274.
- ⁹ Coyne JA and HA Orr. 2004. *Speciation* Sinauer Associates. Sunderland, MA.