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Native forest replacement changes fruit geometry on *Tristerix corymbosus* (Loranthaceae), a keystone mistletoe

El reemplazo del bosque nativo cambia la geometría de los frutos de *Tristerix corymbosus*, un quintral clave

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RESUMEN

Las condiciones microclimáticas pueden alterar rasgos en las plantas, como la geometría del fruto, que es importante para las interacciones planta-frugívoro. Mediante morfometría geométrica se examinó el efecto del reemplazo del bosque sobre la forma de los frutos de *Tristerix corymbosus*, cuya forma cambió significativamente de alargada a redondeada.

Land use change caused by anthropogenic activities is a major driver of biological change (Chapin III *et al.* 2000, Sala *et al.* 2000). In this sense, forest plantations cover 187 million ha worldwide (Brown 2001) and usually their establishment result from the replacement of preexisting native forests (Echeverría *et al.* 2006, Newbold *et al.* 2015) by monocultures of fast-growing exotic trees (mainly *Pinus* spp. and *Eucalyptus* spp.). Productive lands (either active or abandoned) can hold a subset of generalist / tolerant species (Peh *et al.* 2006), usually favored by the presence of native understory vegetation (Nájera & Simonetti 2009, Fontúrbel *et al.* 2016). Even when some species are able to persist after forest replacement, major structural and functional changes alter the ecological scenario in which those species and their interactions thrive (Stockwell *et al.* 2003, Kinnison *et al.* 2007).

Among those species thriving on degraded lands, we can often find mistletoes. These parasitic plants are usually benefited by habitat degradation as result of large sunlight incidence and increased visitation rates of generalist mutualists (Bowen *et al.* 2009), but such apparent benefit involves less obvious ecological consequences such as changes in fruit traits (Fontúrbel *et al.* 2017a); Fontúrbel & Medel 2017). Those changes can have important effects at the community scale, as variation in mistletoe fruit traits may also alter detection, removal and consumption probabilities by frugivores (Onstein *et al.* 2017), ultimately affecting plant recruitment. Here we focused on the hemiparasitic mistletoe *Tristerix corymbosus* (L.) Kuijt (Loranthaceae), a common species of southern South America. Previously we found that microclimatic conditions (and particularly sunlight incidence, which affects fruit ripening process) on abandoned *Eucalyptus globulus* Labill. plantations favored larger seeds on *T. corymbosus*, but there were no significant changes in fruit size (Fontúrbel & Medel 2017). Additionally, mistletoes showed lower visitation rates at the plantation, compared to the native forest (Fontúrbel *et al.* 2017a). Based on this evidence, we hypothesized that forest replacement will alter *T. corymbosus* fruit shape as result of differences in microclimatic conditions. To test this hypothesis, we used a geometric morphometric approach.

This study was conducted at the Valdivian Coastal Reserve (39º57'S 73º34'W), a 50,000-ha private protected area, managed by The Nature Conservancy (Delgado 2010). This private reserve protects an important fraction of the Valdivian rainforest, ecosystem considered as a biodiversity hotspot due to its high proportion of endemic species (Mittermier *et al.* 2005). This Reserve comprises a complex habitat mosaic that consists of native forest and abandoned *Eucalyptus globulus* plantations. The native forest is dominated by *Nothofagus nitida* (Phil.) Krasser, *N. pumilio* (Poepp. & Endl.) Krasser, *Fitzroya cupressoides* I.M.Johnst., *Laurelia philippiana* Looser, *Lomatia ferruginea* (Cav.) R.Br., and *Mitraria coccinea* Cav.; whereas the abandoned plantation is dominated by the exotic tree *Eucalyptus globulus,* plus many understory native plants, being *Rhaphithamnus spinosus* (Juss.) Moldenke, *Aristotelia chilensis* (Molina) Stuntz, *Lapageria rosea* Ruiz & Pav. and *Chusquea quila* Desvau the most abundant ones (Fontúrbel *et al.* 2015). We found *T. corymbosus* in both habitats, parasitizing different native host species (Fontúrbel *et al.* 2017b), but not *E. globulus*. At the temperate rainforests of southern South America (i.e., southwards 37ºS) *T. corymbosus* is solely dispersed by *Dromiciops gliroides*, a frugivorous arboreal marsupial (Amico & Aizen 2000), relying on this species to safely deposit seeds on hosts' branches. Despite frugivorous birds such as *Mimus thenca* (legitimate disperser of many *Tristerix* species) are present at the study area, a fruit color polymorphism is likely to be precluding them to consume *T. corymbosus* fruits at the temperate forests, as ripe fruits remain green –and therefore, undetectable by birds because of the lack of chromatic contrast– due to different sunlight exposure (Amico *et al.* 2011).

During the 2012-2013 austral summer, we sampled 70 adult mistletoes. We collected five ripe fruits from each mistletoe, which were photographed within 6 h from collection. Fruits photos were taken at the same light conditions, at a distance of 20 cm from a flat surface, holding camera perpendicularly. We used a Panasonic Lumix DMC-FZ50 camera (Panasonic Co, Newark NJ). Also, we obtained UTM coordinates of the sampled mistletoes using a Garmin GPS Map 62s device (Garmin, Lenexa KS) with a ≤ 3 m error, measured the amount of light received by each plant using a handheld luxometer, and, registered the host species parasitized by each sampled mistletoe. To characterize the environment surrounding each mistletoe we quantified the proportion of native habitat in a 50-m radius, using aerial photographs and base cartography of the Valdivian Coastal Reserve, as described by Fontúrbel *et al.* (2015).

To examine fruit shape on the sampled mistletoes we used a geometric morphometric analysis based on contours, consisting on four sequential steps: [1] Optimize fruit pictures in order to get binary images (black fruits on a white background) to optimize shape recognition. Some fruits were excluded from the analysis due to blurry edges or unusual irregularities, resulting in a total of 61 plants and 253 fruits included in the analysis. Fruit optimization was conducted using Adobe Photoshop CS6. Fruits of the same individual were saved together in one bitmap file. [2] We used the SHAPE v1.3 (Iwata & Ukai 2002), a software package for assess quantitatively biological shapes, based on elliptic Fourier descriptors (EFD hereafter). SHAPE is composed of four independent analysis modules. First, we used the ChainCoder module to smooth shapes, give a unique ID to fruits within individuals, and obtain the chain code based on the contours of the fruits, which necessary for the following step. Then, we used the Chain2Nef module to perform a Fourier elliptic transformation (based on the chain code generated) to obtain the EFDs. This kind of transformation is recommended for closed shapes that resemble an ellipse (Kuhl & Giardina 1982), like *T. corymbosus* fruits. We used the normalization based on the first harmonic ellipse, using 20 harmonics. We aligned shapes to their larger axis, fruits were oriented to the left in all cases. [3] Once we had calculated the EFDs, we used the PrinComp module to obtain principal components from the normalized Fourier descriptors to summarize and make them easier to analysis (Rohlf & Archie 1984). Principal components were calculated based on the variance-covariance matrices. We extracted the principal components generated altogether with their scores, necessary for conducting further statistical analyses. [4] We used the PrinPrint module to obtain a graphical visualization of the shape variation explained by each principal component.

To assess the effect of habitat replacement and luminosity of fruit shape, we used the first two principal components resulting from the geometric morphometric analysis (explaining 71.07% of the variance) as dependent variables. We fitted spatially explicit Generalized Additive Mixed Models (GAMM), using native habitat proportion, luminosity (values were standardized to mean 0 and variance 1 to avoid large estimate figures in the models) and host species as linear fixed parameters, geographic coordinates were included as a spline term (using a cubic spline smoother), and plant ID as a random factor to account for intra-individual variability (Zuur *et al.* 2009, Wood 2017). We used a Gaussian error distribution; models were fitted using a restricted maximum likelihood approach. We fitted GAMMs using the gamm4 package (Wood & Scheipl 2014) in R 3.4.2 (R Development Core Team 2017). We generated seven candidate models, which were compared by AICc and BIC to select the one with largest explanatory power. Original data, R script, detailed multi-model selection information, and raw SHAPE outputs associated to this paper are available at the *figshare* repository (https://doi.org/10.6084/m9.figshare.5830668).

We obtained 77 principal components from the EFDs of the contour analysis performed, being the first nine components significant. Those nine components explained 92.33% of the fruit shape variance. We kept the first two principal components, explaining 71.07% of the variance (PC1 60.45% and PC2 10.62%), to explain between-habitat shape differences. The PC1 captured the elliptical variation (rounded to elongated fruit shapes), whereas the PC2 captured the shape variance at fruit's tip (Figure 1a). From the seven candidate models fitted (details are available on the *figshare* repository) the model with the best explanatory power was the one including native habitat proportion only. Examining the effects of forest replacement on fruit shape, we found a significant effect of the amount of the remaining native habitat on PC1 (GAMM estimate 0.032 ± 0.015, P = 0.030) but found no effect on PC2 (GAMM estimate -0.001 ± 0.005, P = 0.795; Figure 1b). These results show that fruits are more rounded at the plantation (i.e., negative PC1 values) but elongated at the native forest (positive PC1 values). We found no effects of luminosity (P ≥ 0.465), host species (P ≥ 0.112) and the spatial structure (P ≥ 0.295) in any case. Residual variance of the random factor (plant

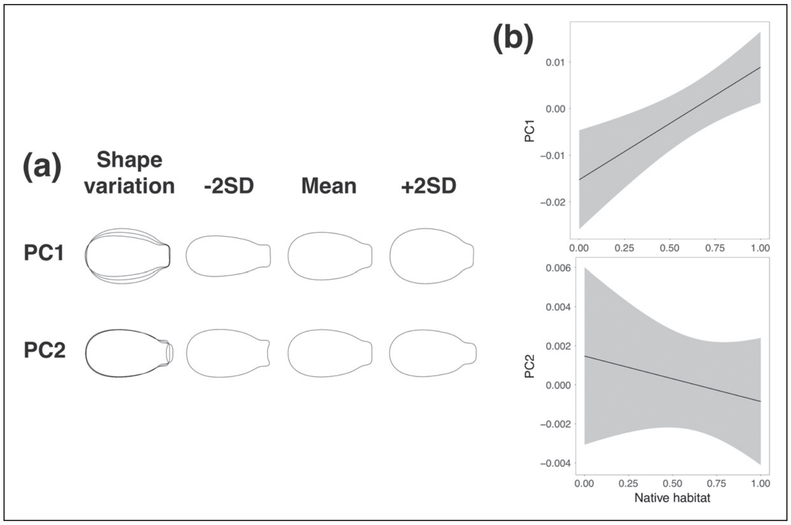

FIGURE 1. (a) *Tristerix corymbosus* shape variation along the first two principal components (PC1 and PC2). Mean shape ± two standard deviations are shown. (b) Relationships between shape components (PC1 and PC2) and the proportion of native habitat (mean ± 95% confidence intervals are show)./ (a) Variación de la forma de *Tristerix corymbosus* a lo largo de los dos primeros componentes principales (PC1 y PC2). Se muestra la forma promedio ± dos desviaciones estándar. (b) Relaciones entre los componentes de forma (PC1 y PC2) y la proporción de hábitat nativo (se muestra la media ± el intervalo de confianza al 95%).

ID) was < 0.001 in all cases (detailed results are available on the *figshare* repository). Native habitat proportion and luminosity were not correlated (Pearson's correlation r = -0.060, P = 0.952).

We found a significant shape variation between the native forest and the abandoned *E. globulus* plantation. Despite *T. corymbosus* is able to persist in the habitat dominated by exotic trees, it is experiencing phenotypic changes. Previously, Fontúrbel and Medel (2017) reported larger seeds at the plantation, but no significant changes were observed in fruit size between habitats. Fruit size largely varied among mistletoes along the study area but given that contour-based geometric morphometric analysis is independent of size, it would not affect our results. Then, lower *D. gliroides* visitation rates were reported at the plantation, but feeding time spent on each mistletoe was up to three times larger than into the native forest (Fontúrbel *et al.* 2017a; 2017b). This evidence suggest that phenotypic changes caused by habitat replacement can affect other processes at the community level, such as seed dispersal, as fruit shape is an important factor determining plant-frugivore interaction, altering detection, removal and consumption probabilities (Onstein *et al.* 2017), which largely determine plant recruitment (Jordano 2000).

Microclimate conditions can alter fruit traits (Galetti *et al.* 2003). In this case, the most likely explanation to the shape variation found was light incidence as abandoned plantations are structurally simpler and have an opener canopy compared to the native forest, being *E. globulus* is the only species above 3 m (the remaining native understory vegetation is dominated by shrubs and small trees). Therefore, they are more exposed to sunlight and have warmer temperatures than the native forest (Fontúrbel & Medel 2017). However, the lack of effect of luminosity on fruit shape variation suggests that the structural modification resulting from forest replacement would alter microclimatic conditions beyond our field measurements (evidenced by the lack of correlation between native habitat proportion and luminosity). Also, our results showed that fruit shape varied irrespectively of the host plant species parasitized, reinforcing the idea of a microclimate influence.

To sum up, the empirical evidence presented here show changes in *T. corymbosus*' fruit shape as result of forest replacement by exotic *Eucalyptus* monocultures. Further, those changes are likely to alter its relationship with the sole seed disperser, the relict marsupial *D. gliroides*. Based on these preliminary results, we encourage future studies aimed at examining phenotype changes in detail, as we live in a changing world where the ecological scenario is more dynamic than ever, breaking the ground for many species but also for the ecological interactions occurring among them.

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