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TALITA SOARES REIS

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Orientador: Prof. Dr. Flavio Antonio Maës dos Santos

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RESUMO

Gradientes de altitude correspondem a intensas variações ambientais em curtas distâncias geográficas servindo como laboratórios naturais para o estudo das respostas das plantas a diferentes condições ecológicas, uma importante iniciativa para prever as reações destes organismos às mudanças ambientais. *Bathysa australis* (Rubiaceae) é uma espécie arbórea amplamente distribuída ao longo do gradiente altitudinal da Serra do Mar cujas populações das partes superior (Floresta Ombrófila Densa Montana, 1010-1100 m) e inferior do gradiente (FOD Submontana, 80-216 m) foram escolhidas como o nosso modelo para avaliar as consequências demográficas e genéticas da altitude nas populações de plantas. Nosso objetivo foi responder às perguntas: (1) A dinâmica populacional de *B. australis* é influenciada pela altitude? (2) Além dos fatores locais, a dinâmica populacional de *B. australis* também é governada por fatores regionais? Ou seja, a dispersão entre populações é um fator determinante na dinâmica local de cada população? (3) Existe estruturação genética nas populações de *B. australis* entre e dentro das fitofisionomias FOD Montana e Submontana? (4) Como se dá a distribuição espacial da variabilidade genética entre as fitofisionomias Montana e Submontana? Estimativas indiretas do fluxo gênico histórico demonstraram que, mesmo num contexto de floresta contínua, as populações de *B. australis* localizadas nos extremos do gradiente estavam isoladas a uma distância inferior a 7 km, o que não foi atribuído à distância geográfica, mas sim à diferença altitudinal. Dois agrupamentos genéticos foram então reconhecidos (Submontana e Montana), indicando ausência de dispersão entre eles de modo que a escala local parece ser suficiente para entender os processos dinâmicos desta espécie. Foram verificadas ainda diferenças nos padrões ecológicos entre as populações das diferentes altitudes. *B. australis* apresentou considerável plasticidade demográfica, pois diferentes estratégias foram reconhecidas nas populações Montana e Submontana, sendo ambas bem-sucedidas ($\lambda > 1$). A relativa estabilidade demográfica observada na população Montana foi substituída por uma dinâmica acelerada com maiores taxas de crescimento e recrutamento na população Submontana. Ainda que ambas estratégias sejam bem-sucedidas, aponta-se para uma pequena superioridade do desempenho de *B. australis* na área Submontana ($\lambda = 1{,}084$) em relação à Montana ($\lambda = 1{,}022$), resultado que está de acordo com os padrões de diversidade genética. Além de maior riqueza alélica, a população Submontana demonstrou maior quantidade de alelos raros, sugerindo que o ambiente de maior altitude provavelmente corresponde a um ambiente mais seletivo. A variação altitudinal encontrada na

Serra do Mar pode ser considerada, portanto, como uma barreira para as populações de *B. australis*, criando assim um cenário de pressão adaptativa às condições impostas pelo gradiente. Nosso estudo revelou que uma espécie de distribuição ampla pode se perpetuar em ambientes climaticamente diversos como os extremos de um gradiente altitudinal nos trazendo perspectivas menos pessimistas em relação a cenários futuros, ao menos no que concerne a estas espécies dotadas de ampla distribuição e plasticidade demográfica. Por outro lado, é necessário considerar o isolamento e as diferenças entre populações localizadas em diferentes altitudes quando na tomada de decisões que visem à conservação das espécies e também à restauração ecológica.

Palavras-chave: altitude, diversidade genética, fitness, fluxo gênico, modelos de projeção integral, taxa de crescimento populacional.

ABSTRACT

Elevation gradients represent steep environmental gradients over short geographic distances, and are considered as natural laboratories for the study of plant responses to variation in ecological conditions that can contribute with forecasting population responses to environmental changes. *Bathysa australis* (Rubiaceae) is a widespread tree along the altitudinal gradient of the Serra do Mar mountain range, SE Brazil, whose upper (1010-1100 m) and lower (80-216 m) populations of the gradient were chosen as our model to evaluate the demographic and genetic consequences of elevation in plant populations. We aimed to answer the questions: (1) Is there an influence of elevation on *B. australis* demography? (2) In addition to local factors, is *B. australis* demography also driven by regional factors? That is, the migration among populations is an important factor in the local dynamics of each population? (3) Is there a genetic structure among and within upland and lowland *B. australis* populations? (4) How the genetic variability of *B. australis* is spatially distributed among upland and lowland sites? Indirect estimates of historical gene flow have shown that, even within a continuous forest landscape, *B. australis* populations located marginally in the elevation gradient were isolated at less than 7 km apart, which was attributed to the altitudinal difference rather than the geographical distance. Two genetic clusters were then recognized (upland and lowland), indicating absence of migration between them so that the local scale processes seem to be enough to fully understand the dynamics of this species. I also found differences in ecological patterns among populations of different altitudes. *B. australis* showed considerable demographic plasticity, as different strategies were displayed by upland and lowland populations, both successful since they keep populations above the substitution rate ($\lambda > 1$), yet demonstrating the influence of altitude on *B. australis* population dynamics. The relative demographic stability observed in the upland contrasted with fast dynamics with higher growth and recruitment rates in the lowland. Even that both strategies were successful, *B. australis* performed slightly better in the lowland ($\lambda = 1.084$ [1.040, 1.101]) than in the upland site ($\lambda = 1{,}022$ [1.011, 1.030]), a result consistent with the intrapopulational genetic diversity patterns found. Besides greater allelic richness, the lowland population also demonstrated greater amount of rare alleles, suggesting that the upland site may be more selective. The elevational variation found in the Serra do Mar mountain range can therefore be considered a barrier to *B. australis* populations, thus creating a potential scenario for adaptation to the different conditions imposed by the elevation gradient. My results have

shown that a widely distributed species can perpetuate itself in a wide range of environmental conditions such as the climatic extremes along an elevation gradient, suggesting a less pessimistic view of future scenarios of climate change, at least with respect to widespread species with demographic plasticity. On the other hand, it is important to consider the isolation and the existing differences among populations located at different elevations when making decisions regarding species conservation as well as ecological restoration.
Keywords: altitude, fitness, gene flow, genetic diversity, Integral Projection Model, population growth rate.

SUMÁRIO

INTRODUÇÃO GERAL

Populações de plantas em florestas tropicais estão sujeitas a níveis variados de heterogeneidade do ambiente que, em última análise, influenciam suas taxas demográficas, tais como reprodução, crescimento e mortalidade. Com isso, a distribuição dos indivíduos no espaço reflete, em grande parte, a distribuição espacial dos recursos, principalmente luz, água e nutrientes (Harper 1977). Deste modo, a abundância de uma dada espécie em um local e momento precisos será fruto de suas taxas demográficas, tanto atuais quanto passadas, ou seja, será o saldo dos fatores locais contemporâneos e históricos que promoveram os nascimentos e as mortes. Por fatores locais entendemos como sendo não só o ambiente físico, com suas condições (e.g. temperatura) e recursos (e.g. nutrientes), mas também as interações bióticas que atuam nesta escala.

No entanto, outros processos que atuam além da escala local podem responder pela abundância das espécies, tais como a imigração e a emigração. Estes processos, em geral, são negligenciados nos estudos populacionais, que contemplam apenas a escala local avaliando a influência de fatores locais na demografia (Eriksson 1996). No âmbito regional, a heterogeneidade espacial pode levar ao isolamento de populações, caracterizando assim, populações distintas ou mesmo uma metapopulação. Neste caso, o isolamento seria parcial formando então um sistema de populações locais (ou sub-populações) separadas no espaço, porém unidas pelo fluxo de indivíduos (Hanski & Gilpin 1991). Neste contexto, em que as populações são espacialmente estruturadas, a quantificação das taxas de imigração e emigração é fundamental, uma vez que, para compreender a dinâmica de uma população local na sua totalidade é importante entender a dinâmica das populações vizinhas (Husband & Barret 1996). Com isso, a investigação dos processos em escala regional torna-se importante para a definição da unidade de dinâmica a ser considerada.

No caso das populações de plantas, o que une a demografia destas na paisagem é principalmente o fluxo de sementes, embora o fluxo de pólen também desempenhe um papel importante. É especificamente a dispersão de sementes por longas distâncias que atua conectando populações espacialmente isoladas. No entanto, este tipo de dispersão é caracteristicamente raro e, dependendo do modo pelo qual a planta dispersa suas sementes, pode ser ainda mais raro. Plantas cujos propágulos são dispersos pela fauna ou pelo vento têm relativamente mais chances de atingir longas distâncias de dispersão do que plantas do tipo autocóricas, que prescindem de vetores, fazendo sua própria dispersão. No entanto, a

dispersão por longas distâncias, devido a sua raridade, é difícil de ser quantificada (Cain et al. 2000). Métodos moleculares de estimativa indireta da dispersão têm sido atualmente utilizados (Ouborg et al. 1999; Cain et al. 2000), tornando possível incorporar este aspecto nos estudos de dinâmica populacional.

A dispersão de pólen e sementes possui grande importância não apenas para os processos demográficos, mas também para os genéticos, uma vez que promovem o fluxo gênico entre populações isoladas no espaço. A intensidade desse fluxo é determinante para a distribuição espacial da variabilidade genética destas populações, pois uma vez que não há troca gênica, as populações tendem a se tornar mais distintas, ou seja, mais estruturadas, e sofrer reduções na sua variabilidade intrapopulacional. Isso pode acontecer, por exemplo, em plantas cuja dispersão é restrita, tal como em espécies autocóricas. Sendo assim, a quantificação deste fluxo é de grande relevância quando se busca compreender a distribuição da variabilidade genética entre e dentro de populações para o posterior aproveitamento destas informações na conservação e definição de estratégias de manejo de populações naturais.

Estudos avaliando as consequências demográficas e genéticas do isolamento de populações geralmente são restritos a paisagens antropizadas onde existe uma clara fragmentação do habitat. No entanto, paisagens de vegetação contínua podem representar situações suficientemente heterogêneas para interromper o fluxo tanto de indivíduos quanto de genes. Os gradientes de altitude gerados por formações montanhosas, por exemplo, são casos especiais de variações de clima, e muitas vezes também de solo, que podem representar verdadeiras barreiras para as populações de plantas nele distribuídas com implicações não apenas demográficas como também genéticas (Grubb & Whitmore 1966, Grubb 1977, Gentry 1988, Herrera & Bazaga 2008, Lieberman et al. 1996, Byars et al. 2007, 2009, Shi et al. 2011).

Os gradientes de altitude na diversidade e estrutura genética de populações naturais

Gradientes de altitude são elementos que agregam significativa variação a paisagem e que podem influenciar tanto a diversidade quanto a estrutura genética das populações, particularmente das populações localizadas marginalmente nos limites de distribuição superior e inferior do gradiente (Herrera & Bazaga 2008, e.g. Byars et al. 2009, Shi et al. 2011). Neste tipo de gradiente podemos encontrar uma grande variação de condições ambientais em distâncias curtas, e as populações inseridas nesse contexto podem estar sujeitas a processos de adaptação local (Byars et al. 2007, Shi et al. 2011). Fatores como temperatura, precipitação, características do solo e da comunidade biótica variam de forma acentuada e

provavelmente afetam a qualidade do habitat para uma dada espécie (Grubb & Whitmore 1966, Grubb 1977, Gentry 1988, Lieberman et al. 1996). Além disso, gradientes de altitude podem representar barreiras significativas ao fluxo gênico, dificultando o movimento de polinizadores e dispersores de sementes (Schuster et al. 1989). Byars et al. (2009), estudando a herbácea alpina *Poa hiemata* em três gradientes altitudinais na Austrália, verificaram um baixo fluxo gênico entre as populações das altas e baixas altitudes e atribuiu esse resultado a forte separação fenológica que eles observaram ao longo do gradiente, o que pode ter levado a oportunidades reduzidas para os insetos polinizadores. Esses autores encontraram uma estruturação genética mais forte entre altitudes dentro de transectos do que entre transectos de mesma altitude, mesmo que as distâncias entre transectos tenham sido maiores, indicando que em alguns casos a variação altitudinal pode representar uma barreira mais intensa ao fluxo gênico do que a distância geográfica.

Com isso, pode existir uma grande variação nos padrões de distribuição da diversidade genética entre e dentro de populações ao longo dos gradientes de altitude (Ohsawa & Ide 2008). No que diz respeito à diversidade genética intrapopulacional, enquanto alguns estudos demonstraram picos de diversidade nas altitudes superiores (e.g. Gämperle & Schneller 2002), outros têm encontrado maior diversidade nas baixas altitudes (e.g. Quiroga & Premoli 2007) ou altitudes intermediárias (e.g. Byars et al. 2009). A ocorrência de gargalos populacionais durante a expansão das florestas, por exemplo, tem sido sugerida como responsável pela diminuição da diversidade genética nas regiões mais elevadas de alguns gradientes (Ohsawa & Ide 2008). Este parece ser o caso de *Podocarpus parlatorei* nas montanhas da floresta de Yungas, América do Sul, para a qual o declínio da diversidade genética nas maiores altitudes parece refletir a migração da floresta durante os períodos glaciais (Quiroga & Premoli 2007). Além da presença de gargalos, a reprodução clonal, mais frequente em altitudes elevadas, também pode resultar em reduções na diversidade genética de algumas populações com o aumento da altitude (Ohsawa & Ide 2008).

Por outro lado, a perda de diversidade genética nas populações periféricas e mais isoladas devido a reduções no fluxo gênico e deriva gênica podem gerar picos de diversidade em altitudes intermediárias (Byars et al. 2009). Essa perda de diversidade genética nos extremos altitudinais pode ocorrer ainda devido à seleção natural, no caso dos marcadores estarem ligados a loci sob seleção (Byars et al. 2007, Byars et al. 2009). Existe ainda o padrão de aumento da diversidade com a altitude, que pode estar associado a causas antrópicas (i.e. urbanização) para a perda de diversidade nas baixas altitudes, mas pode estar associado também com a correlação positiva entre heterozigosidade e aptidão em ambientes severos

(David 1998, Ohsawa & Ide 2008). Gämperle & Schneller (2002), por exemplo, sugeriram que o aumento da heterozigosidade de *Cystopteris fragilis* com a altitude poderia ser interpretado como uma adaptação às condições extremas das altitudes elevadas, já que a heterozigosidade estaria correlacionada com características da aptidão individual. Por último, existem relatos que mostram valores constantes de variabilidade genética ao longo de todo gradiente (e.g. Truong et al. 2007, Ohsawa et al. 2008), o que sugere um fluxo gênico livre entre as populações de diferentes altitudes (Truong et al. 2007), mas pode indicar ainda a presença de outros fatores ambientais além da altitude, e.g. topografia, que estejam atuando de maneira preponderante nos padrões de diversidade genética (Ohsawa et al. 2008). Com isso, o processo por trás da formação destes padrões pode ser variável, mas parece que o conjunto de condições ambientais ótimas para uma espécie desempenha um papel importante na concentração da sua maior diversidade genética (Ohsawa et al. 2007, Ohsawa & Ide 2008).

Os gradientes de altitude na demografia de populações naturais

Mudanças ambientais causadas por variações de altitude podem ainda ter fortes implicações para as taxas demográficas das espécies, uma vez que a heterogeneidade ambiental característica destes gradientes é causada pela variação conjunta de diversos fatores. Dentre estes podemos incluir a temperatura, umidade do ar, precipitação, topografia, características químicas e físicas do solo e disponibilidade de luz (Grubb & Whitmore 1966, Grubb 1977). Em parte, isso se deve a ocorrência de neblina nas partes mais elevadas do gradiente, o que aumenta a umidade atmosférica e reduz a radiação solar e a evapotranspiração (Pendry & Proctor 1996). Estas mudanças ambientais possivelmente afetam a qualidade do habitat para as espécies vegetais, especialmente em se tratando de espécies com ampla distribuição, o que pode ser notado avaliando como esses gradientes influenciam os muitos estágios envolvidos no ciclo de vida de uma planta. Nesse sentido, existe uma vasta literatura medindo desde alterações na fenologia e no sucesso reprodutivo das espécies até mudanças nos padrões de crescimento e sobrevivência de indivíduos adultos (e.g. Eriksen et al. 1993, Hemborg & Karlsson 1998, Bühler & Schmid 2001, Fabbro & Körner 2004, Fernández-Calvo & Obeso 2004, Giménez-Benavides et al. 2007, Cierjacks et al. 2008, Brito & Sazima 2012).

Os efeitos da altitude na fenologia das espécies incluem alterações tanto na época de início da floração, quando na intensidade e duração. Tem sido consistente entre os estudos a detecção de atrasos no início da floração com o aumento da altitude (Vera 1995, Blionis & Vokou 2002, Trtikova et al. 2010, Brito & Sazima 2012, Gauzere et al. 2013, Scheepens &

Stöcklin 2013), o que pode ser atribuído a diminuições na temperatura. Observa-se por outro lado, incrementos na intensidade (Totland 1993, Brito & Sazima 2012, Scheepens & Stöcklin 2013) e duração da floração (Fabbro & Körner 2004, Brito & Sazima 2012). Scheepens & Stöcklin (2013) notaram que, embora as plantas de erva *Campanula thyrsoides* transplantadas para altitudes menores dos Alpes Suíços florescessem antes, o número de flores por planta era reduzido. Essa separação fenológica entre as populações de diferentes altitudes, por sua vez, pode ocasionar uma redução no fluxo gênico ao alternar as opções de recurso para os insetos polinizadores (Byars et al. 2009).

Além disso, a abundância de insetos polinizadores nas altitudes mais elevadas parece ser comprometida (Arroyo et al. 1985, Totland 1993), podendo causar assim uma redução no sucesso reprodutivo via limitação de pólen (Eriksen et al. 1993, Totland 1993, Hemborg & Karlsson 1998, Trtikova et al. 2010, Brito & Sazima 2012). Nos Andes do Chile central, por exemplo, observou-se para 134 espécies de plantas uma frequência menor de visitas por insetos nas altitudes maiores, o que foi atribuído a reduções na abundância destes insetos em decorrência das baixas temperaturas (Arroyo et al. 1985). Na região tropical foram obtidos resultados semelhantes na floresta Atlântica da Serra do Mar para a espécie arbustiva *Tibouchina pulchra* (Brito & Sazima 2012). Embora tenha sido encontrada maior intensidade e duração da floração na população de maior altitude, o mesmo incremento não foi observado na produção de frutos. Juntamente com as baixas no sucesso reprodutivo, é possível que haja uma restrição da regeneração nas altitudes elevadas de maneira geral, como já foram constatadas diminuições no número de plântulas (Bühler & Schmid 2001, Cierjacks et al. 2008), o que pode ser atribuído também a uma maior mortalidade das mesmas (Trtikova et al. 2010). Por outro lado, também foram observadas diminuições nas taxas de predação de sementes com o aumento da altitude, levando a incrementos na sobrevivência de sementes (ver Hillyer & Silman 2010). Trabalhando com 24 espécies dos Andes peruanos, os autores concluíram que a redução na predação de sementes não só era uma provável consequência da baixa abundância de predadores como também verificaram que isso teria uma repercussão positiva para fecundidade das espécies das altitudes maiores.

Além de afetar a fecundidade, a variação altitudinal pode apresentar ainda implicações para os padrões de crescimento e mortalidade. Diminuições no crescimento individual com a altitude têm sido frequentemente reportadas (Grant & Mitton 1979, Fernández-Calvo & Obeso 2004, Coomes & Allen 2007, King et al. 2013) e podem ser atribuída a alguns fatores, como redução nas temperaturas do ar e do solo (e.g. Richardson et al. 2005, King et al. 2013), aumento da incidência de ventos, redução na disponibilidade de nutrientes e redução da

estação de crescimento no caso de florestas sazonais (Coomes & Allen 2007). Nos Alpes Suíços, King et. al. (2013) usaram uma abordagem dendrocronológica e observaram menores taxas de crescimento para as espécies *Larix decídua* e *Picea abies* nas altitudes maiores. Como eles verificaram que as populações ao longo do transecto altitudinal eram geneticamente similares, descartaram a possibilidade de adaptação local e atribuíram as variações no crescimento à correlação negativa entre altitude e temperatura.

Ainda nos Alpes Suíços, as baixas temperaturas do inverno provocaram uma alta mortalidade de plântulas de *Erigeron annuus* a 1000 metros de altitude, próximo ao limite altitudinal da espécie (Trtikova et al. 2010). Os autores sugeriram estas baixas temperaturas do inverno como sendo o fator limitante que define o extremo altitudinal da espécie, cuja distribuição está centrada nos 400 metros de altitude. Ao contrário de *E. annuus*, uma espécie característica de baixas altitudes, a espécie alpina *Erysimum capitatum* apresentou menor sobrevivência de todos os estádios nas altitudes inferiores das montanhas rochosas do Colorado (Kim & Donohue 2011). Estes resultados chamam a atenção de novo para o papel das condições ambientais ótimas para cada espécie, ou seja, a posição dentro de um gradiente ambiental em que a espécie encontra a maior parte de suas necessidades ecológicas e consegue se perpetuar (Brown 1984, Eckert et al. 2008).

O nosso sistema: o gradiente altitudinal da Serra do Mar

Na Floresta Ombrófila Densa (FOD) Atlântica da Serra do Mar (SE Brasil), aumentos na altitude também resultam em diferenças na estrutura, composição e riqueza de espécies da comunidade vegetal (Oliveira-Filho & Fontes 2000, Alves et al. 2010, Joly et al. 2012, Scaranello et al. 2012, Eisenlohr et al. 2013, Sanchez et al. 2013). De acordo com o Sistema Nacional de Classificação da Vegetação do IBGE (Veloso et al. 1991), dois tipos de vegetação podem ser facilmente encontrados nesta região por causa da vasta área que ocupam: a FOD Submontana (50 a 500 m de altitude) e a FOD Montana (500 a 1200 m). As populações de espécies de plantas nestes diferentes tipos de vegetação ao longo do gradiente de altitude da Serra do Mar estão sujeitas a situações ambientais muito distintas, uma vez que já foram relatados aumento na ocorrência de neblinas e redução da precipitação e da temperatura do solo com o aumento da altitude (Alves et al. 2010, Souza Neto et al. 2011, Eisenlohr et al. 2012, Joly et al. 2012).

Bathysa australis (A. St.-Hil.) Hook. f. K. Schum ex. (Rubiaceae) é uma árvore tropical endêmica da Floresta Atlântica brasileira, amplamente distribuída ao longo do gradiente altitudinal da Serra do Mar. Por esse motivo, as populações de *B. australis* das

partes superior (FOD Montana) e inferior do gradiente (FOD Submontana) foram escolhidas como o nosso modelo para avaliar as consequências demográficas e genéticas da altitude nas populações de plantas. De maneira geral, nosso objetivo foi responder às seguintes perguntas: (1) A dinâmica populacional de *B. australis* é influenciada pela altitude? (2) Além dos fatores locais, a dinâmica populacional de *B. australis* também é governada por fatores regionais? Ou seja, a dispersão entre populações é um fator determinante na dinâmica local de cada população? (3) Existe estruturação genética nas populações de *B. australis* entre e dentro das fitofisionomias FOD Montana e Submontana? (4) Como se dá a distribuição espacial da variabilidade genética entre as fitofisionomias FOD Montana e Submontana?

Para responder a estas questões o presente estudo foi dividido em três capítulos. No primeiro capítulo fizemos a caracterização dos dez marcadores microssatélite desenvolvidos para *B. australis*, que foram posteriormente utilizados na avaliação da diversidade e estrutura genética das populações desta espécie, tema do segundo capítulo. Neste segundo capítulo, além da altitude, avaliamos ainda o papel da distância geográfica na distribuição espacial da diversidade genética de *B. australis*. Por último, no terceiro capítulo abordamos principalmente como a diferença altitudinal entre as fitofisionomias Montana e Submontana afetou a estrutura e a demografia de *B. australis.* Em segundo plano, abordamos se o histórico de corte seletivo existente em uma das três parcelas de ambas as altitudes teve repercussões para a estrutura e dinâmica das populações de *B. australis*.

A espécie

B. australis é uma espécie arbórea da família Rubiaceae que pertence aos estratos inferiores da floresta, atingindo até 20 metros de altura. É uma espécie endêmica da Floresta Atlântica, um dos hotspots de biodiversidade brasileiro (Myers et al. 2000) por seu elevado grau de endemismo e histórico de ameaçada que ainda persiste nos dias atuais (Tabarelli et al. 2010). As espécies desse gênero são neotropicais ocorrendo predominantemente em formações florestais de encosta entre 600-1100 metros de altitude (Germano-Filho 1999). *B. australis* ocorre predominantemente no Sul e Sudeste do Brasil e, portanto, seu epíteto *australis* faz referência a sua distribuição.

O período de floração de *B. australis* é geralmente de dezembro a abril, enquanto a frutificação costuma ocorrer de fevereiro a junho (Freitas & Andrich 2013). *Bathysa* exibe inflorescências terminais do tipo tirso e suas flores são hermafroditas, homostílicas e auto-compatíveis (Freitas & Andrich 2013). Apesar do sistema de compatibilidade, as flores de *Bathysa* apresentam dicogamia, uma separação temporal das funções masculinas e femininas

que favorece a polinização cruzada em flores hermafroditas (Freitas & Andrich 2013). Os principais polinizadores das flores de *B. australis* são abelhas e vespas sociais (Freitas & Andrich 2013). O fruto é uma cápsula de 4-6 mm de comprimento, e a dispersão de sementes é do tipo autocórica (Ziparro et al. 2005, Colloneti et al. 2009).

Área de estudo

Este estudo foi realizado durante o período de 2010-2012 em duas áreas do gradiente de altitude (80-1100 m) da Floresta Atlântica da Serra do Mar no Estado de São Paulo, sudeste do Brasil (Figura 1). A primeira foi uma área de FOD Montana (1010-1100 m) e a segunda de FOD Submontana (80-216 m), ambas localizadas dentro do Parque Estadual da Serra do Mar, municípios de São Luís do Paraitinga e Ubatuba, respectivamente. Em ambos os locais foram amostradas populações *B. australis* em parcelas de 1-ha já estabelecidas para o projeto "Biota Gradiente Funcional" (ver Joly et al. 2012). No total, seis populações foram amostradas, correspondente a seis parcelas de 1-ha: três na área Submontana (L1, L2 e L3; correspondentes a F, G e H em Joly et al. 2012) e três na área Montana (U1, U2 e U3; correspondentes a K, L e N em Joly et al. 2012). As distâncias entre parcelas dentro de cada área variaram de 100 - 900 m enquanto a distância entre áreas foi de aproximadamente 6 km.

As duas áreas amostradas diferem em clima e precipitação. O clima da região é tropical úmido na área Submontana, enquanto o clima da Montana é subtropical úmido, ambos sem estação seca (Setzer 1966). A temperatura média anual na área Submontana é 22 °C e a precipitação média anual é superior a 2.500 milímetros. Mesmo nos meses mais secos, de junho a agosto, a precipitação média mensal é superior a 60 mm. A temperatura média anual da área Montana é de 20 °C e a precipitação média anual é superior a 1100 milímetros. Nos meses mais secos, de abril a setembro, a precipitação média mensal é superior a 30 mm (EMBRAPA 2003).

Ambas as áreas têm trechos específicos da floresta que foram historicamente explorados para a produção madeireira através de cortes seletivos, sendo relatadas para estes trechos diferenças na estrutura, diversidade e composição da comunidade vegetal (e.g. Padgurschi et al. 2011, Ramos et al. 2011). Na área de FOD Montana, a parcela U3 esteve sujeita a exploração madeireira até 1970 (Padgurschi et al. 2011), enquanto na área de FOD Submontana, a parcela L1 foi explorada até 1982 (Ramos et al. 2011). No entanto, em ambos os casos foram extraídas apenas árvores de grande porte e potencial madeireiro, não incluindo as populações de *B. australis*.

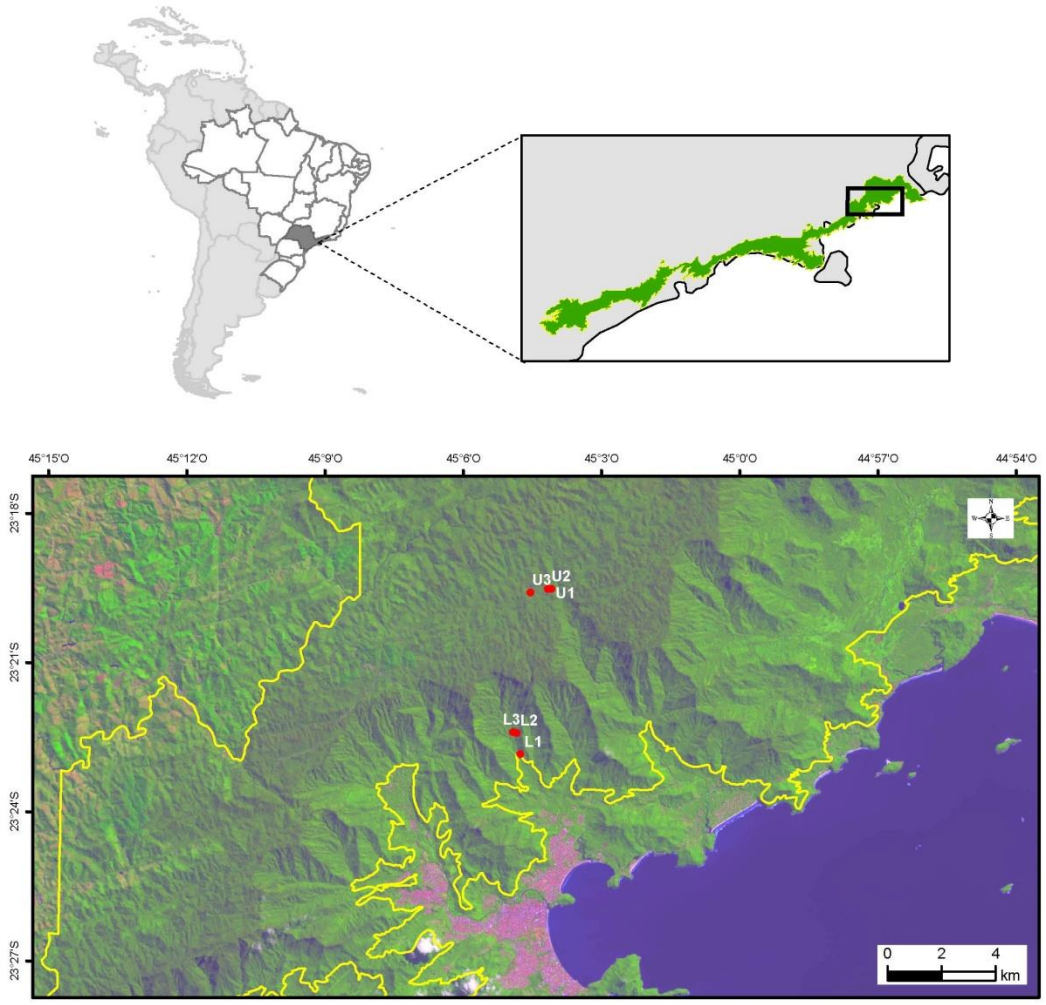

Figura 1. Mapa da Serra do Mar indicando a localização das seis populações de *B. australis*. L1, L2 e L3 correspondem às parcelas da área Submontana (Ubatuba, SP, Brasil), enquanto U1, U2 e U3 correspondem às parcelas da área Montana (São Luis do Paraitinga, SP, Brasil). Todas as parcelas estão dentro dos limites do Parque Estadual da Serra do Mar (linha amarela). Imagem Landsat 8 (654 composição RGB) tirada em abril de 2014. Datum: WGS84 e resolução de 30 metros.

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CAPÍTULO 1

CHARACTERIZATION OF TEN MICROSATELLITE LOCI FOR *BATHYSA AUSTRALIS* (A.ST.-HIL.) K.SCHUM. (RUBIACEAE)[1]

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ABSTRACT

- *Premise of the study*: *Bathysa australis* is a common subcanopy tree from the Atlantic rainforest that is pollinated by bees and wasps and produces autochoric seeds. This species has a great phenotypic plasticity along the elevational gradient of Serra do Mar in Southeastern Brazil. We expect to assess the genetic diversity and gene flow between populations of this species along the elevational gradient.
- *Methods and Results*: We developed a microsatellite-enriched genomic library for *B. australis*, and 10 microsatellite loci were successfully amplified, varying from one to 13 alleles per locus. The observed and expected heterozygosities ranged from 0.333 to 0.900 (0.629 on average) and 0.564 to 0.900 (0.742), respectively.
- *Conclusions*: These are the first microsatellite markers developed for the genus *Bathysa* and may be useful in other species of the Condamineeae tribe. These primers will be an important tool for studies of population ecology and conservation genetics.

Keywords: Atlantic rainforest; conservation genetics; medicinal plant; polymorphism; population ecology

INTRODUCTION

Bathysa australis (A.St.-Hil.) K.Schum. (Rubiaceae) is a subcanopy tree that is widespread through the elevational gradient (100 m up to 1000 m a.s.l) of the Atlantic rainforest of Serra do Mar in São Paulo State, Brazil. This species is a common plant in Atlantic rainforest patches (e.g., Ramos et al., 2011) and has an important role in this ecosystem functioning, e.g. providing nectar resource to a variety of insects (Andrich, 2008). Furthermore, its bark is used by folk medicine (Germano-Filho, 1999), which indicates its social value in addition to its ecological value. *B. australis* displays great phenotypic plasticity in leaf size and color along the elevational gradient, is pollinated mainly by bees and wasps (Andrich, 2008), and presents autochoric seed dispersal (Pedroni, 2001). Because we believe the elevation gradient might function as a barrier for *B. australis* gene flow, the investigation of the spatial distribution of the dispersal, pollination and genetic diversity of this plant could generate exciting information regarding its population biology. Above all, the Brazilian Atlantic rainforest is significantly threatened (Myers et al., 2000), and the

microsatellite tools developed in this study might help to evaluate the impacts and define conservation strategies.

METHODS AND RESULTS

We extracted genomic DNA from leaf tissue samples using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA). A microsatellite-enriched library was then developed following Billotte et al. (1999). We digested the DNA samples with the *Rsa*I restriction enzyme (Invitrogen, Carlsbad, California, USA) for three hours at 37°C, and the resulting fragments were ligated to *Rsa*I adapters for two hours at 20°C. The fragments containing microsatellites were selected by hybridization with $(CT)_8$- and $(GT)_8$-biotinylated oligonucleotides, followed by capture with Streptavidin MagneSphere Paramagnetic Particles (Promega, Fitchburg, WI, USA). The selected DNA fragments were PCR-amplified in 100-µL final volume containing 20µL of selected fragments, 1x PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 4 pmol of primer Rsa21, and 2.5 U of *Taq* DNA polymerase. A PTC-100 thermal cycler (MJ Research, Waltham, Massachusetts, USA) was used with the following program: 95°C for 1 min, followed by 25 cycles of denaturation at 94°C for 40 s, 60°C for 1 min, extension of 72°C for 2 min, and a final extension of 72°C for 5 min. The amplification products were cloned into the pGEM-T (Promega) vector. Plasmids were transformed into *Escherichia coli* XL1-Blue competent cells, and positive clones were selected using the β-galactosidase gene and grown overnight in an HM/FM medium with ampicillin. A total of 96 positive clones were bi-directionally sequenced using an automated ABI PRISM 377 sequencer (Applied Biosystems, Foster City, CA, USA) with T7 and SP6 primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequences were assembled and edited using Seqman (DNAStar, Madison, WI, USA). The repetitive regions were identified using the Simple Sequence Repeat Identification Tool (Temnykh et al., 2001), and 30 primer pairs were designed using WebSat (Martins et al., 2009). Ten primer pairs amplified microsatellite regions and were selected for screening (Table 1). The remaining loci were discarded due to amplification failures or nonspecific amplification patterns. The forward primer for each pair was labeled with fluorochromes (HEX and TET).

The PCR amplifications were performed in a 15-µL volume containing 15 ng DNA, 1x PCR buffer, 0.15 mM each dNTP, 0.8 mM each primer, 0.04% BSA, 1.5 mM $MgCl_2$, and 1 U *Taq* DNA polymerase. A PTC-100 thermal cycler (MJ Research) was used with the following program: 96°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min,

1 min at a specific annealing temperature (*Ta*), and a final extension of 72°C for 5 min. The obtained products were verified by electrophoresis on 3% agarose gels containing 0.1 mg ethidium bromide per ml in 1x TBE buffer and genotyped using 6% denaturing polyacrylamide gels dyed with silver nitrate (Creste et al., 2001). We estimated the allele sizes by comparison to a 10-bp DNA ladder (Invitrogen).

The amplicons were electrophoretically separated using an ABI 377 automated sequencer (Applied Biosystems) with GS500 TAMRA marker as the size standard (Applied Biosystems). The fragment size and allele identification were determined using GeneMarker V2.2 software (SoftGenetics, State College, PA, USA). Cross-species amplifications were evaluated using other five species from the Rubiaceae family with varying phylogenetic proximity to *B. australis*: *Bathysa mendoncaei* K. Schum., *Bathysa stipulata* (Vell.) C.Presl and *Rustia formosa* (Cham. & Schltdl.) Klotzsch, Condamineeae tribe, Ixoroideae subfamily, and *Rudgea jasminoides* (Cham.) Muell. Arg. (Psychotrieae tribe), and *Coussarea accedens* Müll Arg. (Coussareeae tribe), Rubioideae subfamily (Bremer and Eriksson 2009).

We characterized the preliminary genetic diversity of *B. australis* populations from lowland (45.0806°W, 23.3762°S, Ubatuba, SP, Brazil) and upland (45.0710°W, 23.3259°S, São Luis do Paraitinga, SP, Brazil) Serra do Mar. Descriptive statistics and Hardy-Weinberg equilibrium tests were performed using GenAlEx 6.5 (Peakall and Smouse, 2006). Nine loci were polymorphic in both populations (Table 2) and for these the average number of alleles was 8.6, ranging from 5 to 13 alleles per locus. The observed and expected heterozygosities ranged from 0.333 to 0.900 (0.629 average) and 0.564 to 0.900 (0.742 average), respectively. The fixation index ranged from -0.089 to 0.611, with an average of 0.147. Four loci in the lower population and three loci in the upper population showed significant deviations from the Hardy-Weinberg equilibrium ($P<0.05$). These results indicated some slight excess of homozygotes, which might have resulted from mating between relatives and/or the inbreeding generated by selfing; *B. australis* is a self-compatible species (Andrich 2008). All ten loci amplified successfully in the other *Bathysa* species, but only four primers performed well for *Rustia formosa* (Table 3); all primers failed for *Rudgea jasminoides* and *Coussarea accedens*.

CONCLUSIONS

The microsatellite markers described here are the first developed for the genus *Bathysa* and will be useful for genetic, ecological, and conservation management evaluations.

Cross-species amplifications suggested that some of these loci may be useful at least in other species from the Condamineeae tribe, Ixoroideae subfamily.

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Table 1. Description of ten microsatellite markers developed for *Bathysa australis*.

Primer	Repeat Motif	Primer Sequence (5' - 3')	Ta (°C)	Size Range (bp)	GenBank Accession No.
BA-02	$(CT)_8$	F: CTTGCCAAACTGAGCTTCTG	62	150-180	KF267877
		R: GGTGATGGTGCTCCTCTTTC			
BA-14	$(TC)_7$	F: CAGCAAAGTCCACAGCACA	62	140-200	KF267878
		R: TGCGTGCACGTGTGAGT			
BA-15	$(CA)_9$	F: TCCCATTTTCCTGGTCGT	55	270-300	KF267879
		R: TGGCATCCAAGACTCTGCTA			
BA-16	$(CA)_{11}$	F: TCACAGATCCTACAACAGCACC	55	190	KF267880
		R: AGAAGGAGAACGCAAATACCC			
BA-22	$(AG)_6$	F: CCACAGGTTTGTGTTTGTTCTC	55	330-360	KF267881
		R: GTCCCATTCCTTTCATATTCCA			
BA-24	$(GA)_{30}$	F: ACAGCGAAGCTCACACACAT	55	170-230	KF267882
		R: TCTGTGGAAGAAGAGTGGGAAT			
BA-25	$(AC)_{40}$	F:TGCCCAGTAAATAGGAGAGATTG	55	150-180	KF267883
		R: TTATGCTGCTGGAATGGTATTG			
BA-26	$(CT)_{25}$	F: AGGTGCATTGGAAAGGTATTGA	65	360-400	KF267884
		R: GTTTGAGGCTTTGGACATACATC			
BA-28	$(TG)_7$	F: AGGACTTCCATTTTGTTGGGTA	55	340-400	KF267885
		R: GGGTTTTAATTTCGTGACTTGC			
BA-30	$(CT)_{33}$	F: CTTGAATGCTGCTGGTAAAGC	65	290-370	KF267886
		R: GCATCCTTTTGGACTCAATTTC			

Note: Ta = annealing temperatures

Table 2. Results of initial primer screening of Lower (45.0806°W, 23.3762°S) and Upper (45.0710°W, 23.3259°S) *Bathysa australis* populations. Only polymorphic loci are shown.

Population	Locus	A	A_e	H_o	H_e	F	HWE[a]
Lower	**BA02**	5	2.417	0.450	0.601	0.232	ns
(N= 20)	**BA14**	12	5.270	0.842	0.832	-0.039	ns
	BA15	8	5.369	0.850	0.835	-0.045	ns
	BA22	5	2.222	0.500	0.564	0.091	ns
	BA24	12	2.462	0.500	0.609	0.158	*
	BA25	7	5.026	0.353	0.825	0.559	**
	BA26	11	8.163	0.600	0.900	0.316	ns
	BA28	8	5.755	0.900	0.847	-0.089	*
	BA30	12	6.968	0.333	0.881	0.611	**
Upper	**BA02**	5	2.606	0.600	0.632	0.026	ns
(N=20)	**BA14**	11	6.422	0.647	0.870	0.234	**
	BA15	8	3.404	0.750	0.724	-0.062	ns
	BA22	6	2.548	0.550	0.623	0.095	ns
	BA24	11	4.938	0.800	0.818	-0.003	ns
	BA25	7	4.040	0.750	0.772	0.003	ns
	BA26	13	7.407	0.650	0.887	0.249	**
	BA28	5	3.162	0.450	0.701	0.342	***
	BA30	10	4.396	0.800	0.792	-0.036	ns

Note: N = number of individuals sampled; A = number of alleles; A_e = effective number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; F = fixation index; HWE = Hardy-Weinberg Equilibrium tests.

[a]Significant departures from HWE are indicated at the following levels: * = 0.05, ** = 0.01, and *** = 0.001, ns = non significant.

Table 3. Results from the cross-amplification tests using primers designed for *Bathysa australis*.

Species	Locus									
	BA02	BA14	BA15	BA16	BA22	BA24	BA25	BA26	BA28	BA30
Bathysa stipulata	+	+	+	+	+	+	+	+	+	+
Bathysa mendoncaei	+	+	+	+	+	+	+	+	+	+
Rustia formosa	+	-	-	-	+	-	-	+	-	+
Coussarea accedens	-	-	-	-	-	-	-	-	-	-
Rudgea jasminoides	-	-	-	-	-	-	-	-	-	-

Appendix 1. Voucher information for species used in this study.

Species	Voucher specimen accession no.	Collection Locality
Bathysa australis (A.St.-Hil.) K.Schum.	HRCB 60163	Rio Claro, SP
Bathysa stipulata (Vell.) C.Presl	HRCB 60107	Rio Claro, SP
Bathysa mendoncaei K. *Schum.*	HRCB 59786	Rio Claro, SP
Rustia formosa (Cham. & Schltdl.) Klotzsch	HRCB 59785	Rio Claro, SP
Coussarea accedens Müll Arg.	HRCB 59788	Rio Claro, SP
Rudgea jasminoides (Cham.) Muell. Arg.	IAC 49279	Campinas, SP

CAPÍTULO 2

Elevation as a barrier: genetic structure for an Atlantic rain forest tree (*Bathysa australis*) in the Serra do Mar mountain range, SE Brazil[**]

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Running title: genetic structure of a forest tree

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Abstract: Distance and discrete geographic barriers play a role in isolating populations, as seed and pollen dispersal become limited. Nearby populations without any geographic barrier between them may also suffer from ecological isolation driven by habitat heterogeneity, which may promote divergence by local adaptation and drift. Likewise, elevation gradients may influence the genetic structure and diversity of populations, particularly those marginally distributed. *Bathysa australis* (Rubiaceae) is a widespread tree along the elevation gradient of the Serra do Mar, SE Brazil. This self-compatible species is pollinated by bees and wasps and has autochoric seeds, suggesting restricted gene dispersal. We investigated the distribution of genetic diversity in six *B. australis* populations at two extreme sites along an elevation gradient: a lowland site (80-216 m) and an upland site (1,010-1,100 m.a.s.l.). Nine microsatellite loci were used to test for genetic structure and to verify differences in genetic diversity between sites. We found a marked genetic structure on a scale as small as 6 km (F_{ST} = 0.21) and two distinct clusters were identified, each corresponding to a site. Although *B. australis* is continuously distributed along the elevation gradient, we have not observed a gene flow between the extreme populations. This might be related to *B. australis* biological features and creates a potential scenario for adaptation to the different conditions imposed by the elevation gradient. We failed to find an isolation-by-distance pattern, although on the fine-scale all populations showed spatial autocorrelation until ~10-20 m. Elevation difference was a relevant factor though, but we need further sampling effort to check its correlation with genetic distance. The lowland populations had a higher allelic richness and showed higher rare allele counts than the upland ones. The upland site may be more selective, eliminating rare alleles, as we did not find any evidence for bottleneck.
Keywords: altitude, gene flow, genetic diversity, microsatellites, Rubiaceae.

Introduction

The problem of speciation in tropical rain forests has long intrigued ecology researchers (Federov 1966). Tropical forests brought attention to the fact that closely related species occur side by side, challenging the ideas of speciation by geographic isolation (Federov 1966). Given a geographic barrier, gene flow interruption was recognized as the primary step towards reproductive isolation (Mayr 1963), and the evolutionary mechanisms behind it could be drift, natural selection, or both. In the absence of a geographic barrier, we would expect unrestricted gene flow and a homogeneous distribution of genotypes in a population. However, even without any discrete barrier, geographic distance may play a role in isolating populations, hampering the movement of alleles among them (Wright 1943;

Hardy & Vekemans 1999). Thus, more distant populations are expected to have less genetic exchange than nearer populations, as a function of limited pollen and seed transport across space. This generates a spatial genetic structure resulting from local genetic drift. Therefore, the set of pollinators and seed dispersal vectors influence the degree of genetic isolation (Loveless & Hamrick 1984).

However, nearby populations with no discrete geographic barrier between them may also suffer from ecological isolation driven by habitat heterogeneity, which may promote population divergence by local adaptation and drift as well (Linhart & Grant 1996; e.g. Antonovics 1971; Misiewicz & Fine 2014). Misiewicz & Fine (2014) found evidence for ecological divergence in an Amazonian tropical tree, *Protium subserratum*, across a mosaic of soil types. They pointed out higher levels of genetic differentiation between adjacent populations in different soil types than between geographically distant populations in the same soil type.

In addition to soil type, other ecological features can interfere with the drift-gene flow balance across the landscape and produce a genetic structure within a species. In this context, elevation gradients, typical of mountain ranges, are special cases of landscape variation that influence both the genetic structure and the diversity of populations, particularly those located marginally at the upper and lower distributional limits (Herrera & Bazaga 2008; e.g. Byars *et al.* 2009; Shi *et al.* 2011). In this type of gradient, we can find a wide variation in environmental conditions over short distances, and the populations experiencing it could be subject to local adaptation (Byars *et al.* 2007; Shi *et al.* 2011). Factors such as temperature, precipitation, soil characteristics, and community composition vary sharply and probably affect habitat suitability for a species (Grubb & Whitmore 1966; Grubb 1977; Gentry 1988; Lieberman *et al.* 1996). Besides, elevation gradients might represent significant barriers to gene flow, hindering the movement of pollinators and seed dispersers (Schuster *et al.* 1989). Byars *et al.* (2009) attributed the low gene flow between high and low altitudes they observed for the Alpine grass *Poa hiemata* to the phenological separation along the altitudinal gradient, which led to reduced opportunities for insect pollinators. These authors found a stronger genetic structuring between altitudes within transects than between transects, even though the distances between transects were larger.

Thus, elevation gradients display a remarkable variation in the distribution patterns of genetic diversity within and between populations along them (Ohsawa & Ide 2008). Concerning within-population genetic diversity, while some studies have demonstrated diversity peaks on higher slopes (e.g. Gämperle & Schneller 2002), others have found greater

diversity at lower (e.g. Quiroga & Premoli 2007) or intermediate elevations (e.g. Oyama *et al.* 1993). In addition to these three patterns, there are also reports showing constant values of genetic variability all over the gradient (e.g. Truong *et al.* 2007), suggesting a free gene flow among populations from different altitudes. The process behind all these patterns can be variable, but it seems that the optimal environmental conditions for a species play an important role in concentrating its major genetic diversity (Ohsawa *et al.* 2007; Ohsawa & Ide 2008). Plant populations in tropical forests are subject to varying levels of environmental heterogeneity, which ultimately influence their survival and reproduction, so the spatial distribution of individuals is mostly related to the spatial distribution of resources and conditions (Harper 1977). Thereby, given a geographical gradient of environmental conditions, a species local abundance might become higher when near its optimal environmental conditions, decreasing gradually as it departs from it (Brown 1984; Eckert *et al.* 2008).

Bathysa australis (A. St.-Hil.) Hook. f. ex K. Schum. (Rubiaceae) is a tropical tree endemic to the Brazilian Atlantic forest, widely distributed along the altitudinal gradient of the Serra do Mar mountain range, Southeast Brazil. For this reason, together with *B. australis* biological features, we believe this species may serve as a model organism to study the effect of altitude on genetic structure and diversity. *B. australis* displays great phenotypic variability in leaf attributes between lowland and upland populations at this gradient (*personal observation*) and is mainly pollinated by bees and wasps (Freitas & Andrich 2013) with an autochoric seed dispersal mode, that is, no known vector disperses their seeds. Such characteristics may indicate a restricted gene flow between these extreme populations and the possibility of an ongoing local adaptation to contrasting environmental conditions.

The flowers of *B. australis* are protogynous, that is, their stigmas become receptive before the opening of anthers (Freitas & Andrich 2013). This lag between stigma reception and anther opening, associated with the earlier flowering of lower populations in the Serra do Mar mountain range (*personal observation*), could generate an upward pollen flow. Because the stigmas from a tree at a given altitude will be receptive earlier than pollen donors are available at the same altitude, the outcrossed pollen pool of this tree might be partly composed of migrant pollen from lower altitudes (Gauzere *et al*. 2013). This increased contribution from outside pollen to upper populations may enhance their genetic diversity and reduce selfing rates.

Bathysa species are usually distributed along forest slopes between 600 and 1,100 m.a.s.l. (Germano-Filho 1999), where we can find typical Montane Tropical Forests (Veloso

et al. 1991). Even though *B. australis* can be found both on higher and lower slopes (Oliveira-Filho & Fontes 2000), we observed that the highest estimated abundances (> 10 indiv.ha^{-1}) are on the upper slopes (e.g. Arzolla 2002; Leite & Rodrigues 2008; Padgurschi *et al.* 2011; Pereira 2011; Sanchez *et al.* 2013), whilst the lowest densities are recorded on the lower ones (*e.g*. Moreno *et al.* 2003; Assis *et al.* 2011; Campos *et al.* 2011; Gomes *et al.* 2011; Prata *et al.* 2011; Sanchez *et al.* 2013), with rare exceptions (e.g. Gomes *et al.* 2011; Ramos *et al.* 2011). For this reason, we believe that the conditions found on the higher slopes are more favorable to the spreading of *B. australis* populations than the conditions found on the lower slopes, which would affect the species' genetic diversity.

In this study, we aimed to investigate within- and between-population genetic structure and diversity for *B. australis* at two extreme sites in the altitudinal gradient of the Serra do Mar mountain range, Southeast Brazil. We addressed the following three questions: (1) Is there a fine-scale spatial genetic structure within *B. australis* populations, as would be expected, considering *B. australis* dispersal mode?; (2) On a larger scale, is there a genetic structure between the upper and lower *B. australis* populations of the Serra do Mar mountain range?; (3) Are the upper mountain populations more genetically diverse than the lower ones?

Materials and Methods

Study species

B. australis is a tree species of the Rubiaceae family that belongs to the lower strata of the forest, reaching up to 15 meters in height. It is an endemic species of the threatened Atlantic rain forest, distributed predominantly in South and Southeast Brazil (Germano-Filho 1999). Its flowering period is usually from December to April, and fructification occurs from February to June (Freitas & Andrich 2013). *Bathysa* displays terminal thyrsus inflorescences and its flowers are hermaphrodite, homostylous, and self-compatible (Freitas & Andrich 2013). In spite of its compatibility system, *Bathysa* flowers are dichogamous, a temporal separation of the male and female functions that may promote outcrossing in hermaphrodite flowers (Freitas & Andrich 2013). The main reward provided by their flowers is nectar, and the key pollinators are social bees and wasps (Freitas & Andrich 2013). The fruit is a capsule of 4-6 mm in length, and seed dispersal is autochoric (Ziparro *et al.* 2005; Colloneti *et al.* 2009).

Study site and sampling design

This study was conducted at two sites along the elevation gradient (80–1,100 m.a.s.l.) of the Atlantic forest of the Serra do Mar mountain range in São Paulo State, SE Brazil (Figure 1). Upland (1,010-1,100 m) and lowland (80-216 m) sites are within Serra do Mar State Park in the municipalities of São Luís do Paraitinga and Ubatuba, respectively. At both sites, *B. australis* populations were sampled in 1-ha plots, already established for the project "Biota Gradiente Funcional" (see Joly *et al.* 2012). In total, six populations were sampled, corresponding to six 1-ha plots: three at the lowland site (L1, L2, and L3) and three at the upland site (U1, U2, and U3).

The two sampled sites differ in climate and precipitation. Whilst the lowland regional climate is tropical humid, with no dry season, the upland climate is subtropical humid (Setzer 1966). The lowland mean annual temperature is 22 °C and the average annual rainfall exceeds 2,500 mm. Even in the driest months, from June to August, the average monthly precipitation is above 60 mm. The upland average annual temperature is 20 °C and the average annual rainfall exceeds 1,100 mm. In the driest months, from April to September, the average monthly precipitation is above 30 mm (EMBRAPA 2003).

According to the Brazilian National Classification System for Vegetation (IBGE System; Veloso *et al.* 1991), the upland sites are covered by Montane Dense Ombrophilous Forest and the lowland sites are covered by Submontane Dense Ombrophilous Forest. Indeed, these two sites differ markedly in vegetation structure and composition (Alves *et al.* 2010; Joly *et al.* 2012), but not in soil characteristics (Martins 2010).

A total of 1,751 individuals (1,044 at the lowland site and 707 at the upland site) were found in the six 1-ha plots, accounting for the whole population, that is, seedlings, juveniles, and adults (Table 1). However, in each population, we collected leaf tissue only from *B. australis* individuals bearing reproductive structures that could reliably signal their reproductive status. Then, only 269 individuals were sampled in February and March 2012, 140 individuals at the lowland site and 129 at the upland site. The different sample efforts in each population reflect the abundance of reproductive individuals in each 1-ha plot at the time. These samples were placed in a thermos with ice and immediately frozen at -20 ºC for DNA conservation. In the laboratory, the samples were kept in a biofreezer at -80 °C until DNA extraction.

Laboratory analysis

We extracted genomic DNA from leaf tissue samples using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). The genetic variation of *B. australis* populations was

examined using nine polymorphic SSR loci. All the loci were developed specifically for *B. australis*, and primer-pair sequences and detailed procedures can be seen in Reis *et al.* (2013). PCR amplifications were performed in a 15 μL volume containing 15 ng DNA, 1 × PCR buffer, 0.15 mM each dNTP, 0.8 mM each primer, 0.04% bovine serum albumin (BSA), 1.5 mM MgCl2 , and 1 U Taq DNA polymerase. A PTC-100 thermal cycler (MJ Research) was used with the following program: 96 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 1 min, 1 min at a specific annealing temperature, and a final extension of 72 °C for 5 min. Amplified products were checked by electrophoresis on 3% agarose gels containing 0.1 mg ethidium bromide per milliliter in 1 × TBE buffer. The amplicons were electrophoretically separated using an ABI 3500 automated sequencer (Applied Biosystems) with GeneScan 600 LIZ marker as the size standard (Applied Biosystems). Fragment size and allele identification were determined using the software GeneMarker version 2.2 (SoftGenetics, State College, Pennsylvania, USA).

Linkage disequilibrium and null alleles

We tested for linkage disequilibrium (LD) using FSTAT with a Bonferroni correction for multiple comparisons. The presence of null alleles was checked using the software FreeNA (Chapuis & Estoup 2007).

Descriptive statistics

Descriptive statistics were performed using GenAlEx version 6.5 (Peakall & Smouse 2006), with the following diversity parameters: number of alleles (A), effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F). We also calculated allelic richness using the program FSTAT (Goudet 1995), because this parameter is more appropriate for comparisons among samples of different sizes (Leberg 2002). Hardy-Weinberg Equilibrium (HWE) tests were conducted using GENEPOP version 4.2 (Raymond & Rousset 1995).

Fine-scale spatial genetic structure

The fine-scale genetic structure was investigated in each population at the plot scale using the software SPAGeDi (Hardy & Vekemans 2002). We used the x,y coordinates of individuals in each 1-ha plot to generate a geographic distance matrix between pairs of individuals. The upper limits for our set of six distance classes were 10, 20, 30, 50, 70, and

100 m. The pairwise estimated genetic distance was the kinship coefficient (*Fij*) by Loiselle *et al.* (1995), which does not assume HWE.

Between-population genetic structure

The population structure inference as well as the number of existing genetic clusters and individuals assigned to each cluster were performed using the software STRUCTURE v.2.3 (Pritchard *et al.* 2000), which uses a Bayesian approach, the Markov Chain Monte Carlo (MCMC). STRUCTURE was run with different values for the number of clusters (*K*), varying from 1 to 7 under the admixture model, with no prior population information. To verify the robustness of our results, we performed 20 independent runs per *K* value with 200,000 burn-in periods and 500,000 Markov Chain Monte Carlo iterations. The statistics described by Evanno *et al.* (2005) was used to detect the most likely number of groups (*K*). The *K* value that best represents the structuring of populations can be identified by the peak value of ΔK.

We used a molecular variance analysis (AMOVA, Excoffier *et al.* 1992) to partition genetic variability between sites (elevational bands), between populations, and within populations. These estimates were made using the software GenAlEx 6.5 (Peakall & Smouse 2006). The combined effects of distance and altitude were checked by correlations of genetic differentiation with geographical distance and with altitudinal difference using Mantel tests and partial Mantel tests (Legendre & Legendre 1998). For Mantel tests, the significance of the correlation between two matrices was assessed by the permutation of rows and columns in the second matrix. The significance of the correlations between genetic distance, expressed as F_{ST}, and geographic distance was estimated with 10,000 permutations. Mantel tests within altitudinal groupings were also carried out, but as an individual-based analysis, because of the low number of populations. We used the software PAST (Hammer *et al.* 2001) to calculate the Euclidean distances between individual pairs, and the software R (R Development Core Team 2008), package 'vegan' (Oksanen *et al.* 2013), to perform Mantel and partial Mantel tests.

Between-altitude genetic diversity

Diversity estimates for the upland and lowland sites were compared (by grouping samples from different sampling sites, either upland or lowland) after 10,000 permutations in FSTAT. Furthermore, we counted exclusive and rare alleles (frequency ≤ 0.05) in each locus to complement diversity comparisons between the upland and lowland sites. These

comparisons were made with a Wilcoxon paired test in R (R Development Core Team 2008), as sample sizes among sites were very similar.

Bottleneck

The software BOTTLENECK version 1.2.02 (Cornuet & Luikart 1996) was used to evaluate the hypothesis of historical reduction in effective population size, which would decrease genetic diversity (bottleneck). BOTTLENECK is based on the evidence that recently bottlenecked populations exhibit an excess of gene diversity (heterozygosity) among polymorphic loci. The expected heterozygosity is compared with the expected heterozygosity at mutation-drift equilibrium, given the allele number and the population sample size. If a population has undergone a bottleneck, gene diversity is higher than that expected at mutation-drift equilibrium, because the latter is calculated from the allele number, which drops faster than heterozygosity (Piry *et al.* 1999). Gene diversity was estimated under the two-phase model (TPM), setting 95% of the single-step stepwise mutation model (SMM) and 5% of the infinite allele model (IAM), with a variance of 12 among multiple steps, as recommended for microsatellite loci (Piry *et al.* 1999). Based on 1,000 replications, one-sided Wilcoxon signed rank tests (Luikart *et al.* 1998) were done to evaluate whether the allele frequency distribution deviated significantly from the expected distribution under mutation-drift equilibrium.

Results

Linkage disequilibrium and null alleles

As we have found only one pair of loci showing LD in population L1 and another one in population U2, and the exclusion of these loci produced the same results, we kept them for our analyses. For each locus, the presence of null alleles was confirmed only in the populations of one of the altitudes. Considering that, we did not take any action, assuming that it could reflect a possible genetic structure between altitudes.

Descriptive statistics

In the six populations sampled, the number of alleles per locus ranged from 2.0 to 16.0 (mean 8.5 ± 0.5). Allelic richness ranged from 2.0 to 11.1 (mean 6.9 ± 2.2). The observed heterozygosity ranged from 0.0 to 0.926 (mean 0.578 ± 0.03), and the expected heterozygosity in HWE ranged from 0.381 to 0.888 (mean 0.706 ± 0.02). The fixation index (*F*) fluctuated from -0.208 to 1.0, with a mean of 0.186 ± 0.04 and 72% of positive values. All

populations had at least two loci with a significantly positive *F* value in the Hardy-Weinberg equilibrium test, indicating an excess of homozygotes. The locus BA30 from the L2 population, for example, did not show any heterozygous individual. All fixation index values were significantly positive using a 95% confidence level ($P < 0.05$), indicating deviations from HWE in all six populations (Table 1).

Fine-scale spatial genetic structure

On the local plot scale, all six populations have shown some degree of spatial genetic structure (Figure 2). Both lowland and upland populations exhibited autocorrelation in the first distance class, indicating that up to the range of ~10-20 m nearby individuals are more genetically related than would be expected by chance.

Between-population genetic structure

Although six populations were initially sampled, the Bayesian analysis allowed for the identification of only two distinct genetic groups, because the highest ΔK value was achieved with $K = 2$ (Figure 3). Thus, lowland populations L1, L2, and L3 were grouped in the first cluster (green, Figure 3) and upland populations U1, U2, and U3 in the second cluster (red, Figure 3), indicating a strong genetic structure that seems to be altitude-related. We also noticed a lack of migrants between these two genetic groups, because no individual originally sampled at the lowland site was included in the upland cluster or vice versa. This structure was reinforced by the global $F_{ST} = 0.21$ and by the molecular analysis of variance, which showed greater variance between the upland and lowland sites (18.6%) than between populations (2.8%; Table 2).

Similarly, pairwise F_{ST} values were low between populations from the same altitude and high between populations from different altitudes, although all values were significant (Table 3). The weak genetic structure observed between populations at the same altitude might result from the fine-scale spatial genetic structure. The gene flow among the upland populations was slightly higher than the gene flow among the lowland populations.

We observed a significant positive correlation between genetic and geographic distance ($r = 0.98$, mantel-$P = 0.02$; Figure 4A). However, when the effect of altitude was discounted by the partial Mantel test, this correlation disappeared ($r = -0.38$, mantel-$P = 0.95$). The pattern of isolation by elevation, on the other hand, proved consistent even after discounting the effect of geographic distance ($r = 0.68$, mantel-$P = 0.02$). This result suggests that geographic distance is not a factor influencing the genetic structure among sites. The

elevation distance between sites seems to be a more relevant element. However, in order to confirm the correlation between elevation and genetic distance, we must increase our sampling effort to include *B. australis* populations from intermediate elevations (Figure 4B).

Within the altitudinal groupings, we found a correlation between genetic and geographical distance for the lowland site (r = 0.03, mantel-*P* = 0.01), but not for the upland site (r = -0.027, mantel-*P* = 0.78), suggesting that the strongest structure observed for the lowland site is a result of geographic distance. However, the partial Mantel test indicates that this correlation disappears again when the effect of altitude is discounted (r = 0.028, mantel-*P* = 0.18). The same occurred with the correlation between genetic and elevational distance, so neither site showed an elevation effect (lowland: r = -0.025, mantel-*P* = 0.79, upland: r = 0.016, mantel-*P* = 0.3), which seems reasonable, because of the low altitudinal variation within sites.

Between-altitude genetic diversity

Only the allelic richness differed between the lowland and upland sites (*P* = 0.027). Contrary to our expectations, the lowland populations showed higher allelic richness (A_R = 7.169) than the upland ones (A_R = 6.591; Table 1). In the same way, lowland populations had significantly higher rare allele counts than the upland ones (*P* = 0.011; Table 4). Exclusive allele counts, however, were not significantly different between sites (*P* = 0.13), although the lowland populations had 20 more exclusive alleles than the upland populations (Table 4).

Bottleneck

None of the populations showed an excess of heterozygosity in relation to the expected at mutation-drift equilibrium. Therefore, there is no evidence for a historical bottleneck.

Discussion

Within-population genetic diversity

In natural populations of plants, a heterozygote deficiency at any locus can be driven by two mechanisms. The first is related to the mating system and possible deviations from panmixia. The second derives from the structuring of populations and the fixation of alleles. *B. australis* populations are prone to both mechanisms in the Serra do Mar mountain range. The facultative autogamy of this species, a result of its self-compatible mating system (Freitas & Andrich 2013), favors the occurrence of inbreeding. Although *B. australis* flowers are

dichogamous, temporally separating the male and female functions in a flower, Freitas & Andrich (2013) suggested that geitonogamous crosses might predominate in *B. australis* populations in the Atlantic rain forest of Itatiaia, in the Serra da Mantiqueira mountain range. Besides, the spatially aggregated arrangement of *B. australis* individuals (*unpublished data*) may facilitate the occurrence of crossing events between relatives. The fine-scale spatial genetic structure has shown that nearby individuals are closely related.

Genetic structuring among populations is another way of increasing homozygote frequencies. *B. australis* have shown a restricted gene flow between the lowland and upland populations, which, associated with random genetic drift, might promote the loss of some alleles and the fixation of others in different populations.

Although a heterozygote deficiency may have negative implications on population fitness, as a result of inbreeding depression, and may narrow a species' capacity to cope with environmental changes by the loss of genetic variability (Keller & Waller 2002; Reed & Frankham 2003), it is a common feature in many plant populations (e.g. Hamrick *et al.* 1993; Hull-Sanders *et al*. 2005; Byars *et al.* 2009; Degen *et al.* 2013) and emerges naturally through the mechanisms cited above.

Between-population genetic structure

The strong genetic structuring found between the lowland and the upland populations of *B. australis* in the Serra do Mar mountain range was expected, considering this species' biological features. No dispersal vectors are known for the seeds of *B. australis*, which has been classified as an autochoric species. This means that its seeds travel just a few meters from the mother plant, producing a strong spatial genetic structure. As we have seen, with a distance of ~10-20 m between them, individuals are more genetically similar than would be expected by chance. This feature generates the strong aggregate spatial distribution pattern that has been observed for this species in the Serra do Mar (*unpublished data*). It is thus possible that the restricted seed dispersal can lead to limited pollen dispersal by creating higher local tree densities, increasing the positive correlation between pollen and seed dispersal distances (Hardy *et al.* 2006). Moreover, fine-scale spatial genetic structure is mostly related to seed dispersal limitations, whilst the genetic structure on coarser scales is more related to pollen dispersal (Dick *et al.* 2008), as pollen flow is usually more extensive than seed flow (Petit *et al.* 2005).

B. australis flowers are mostly pollinated by social bees and wasps (Freitas & Andrich 2013), small insects that usually fly over short distances (Dick *et al.* 2008). In closed canopy,

small insects usually do not disperse beyond 300 m (Dick *et al.* 2008), although they can sometimes travel great distances (e.g. Janzen 1971; Dick *et al*. 2003). Despite that, it seems that these insects' abundance is affected by altitudinal variation. Brito & Sazima (2012), working with the shrub species *Tibouchina pulchra* Cogn (Melastomataceae) at the same study site, observed that the availability of pollinators varied considerably with altitude, and that the presence of effective pollinator bees was up to 200 times lower at the upland site in the Serra do Mar.

Animal pollination is strongly influenced by local density and flowering synchrony. High local densities, as we observed for *B. australis* populations at the study site, may restrict pollen dispersal by concentrating the pollinator's resource on a single spot. Besides, synchrony leads to pollinator satiation, causing it not to visit other plants or to visit only very close ones (Dick *et al*. 2008). Furthermore, we observed a considerable asynchrony in the timing of flowering and fruiting phenophases between the upland and lowland *B. australis* populations (*personal observation*), which contributes to a limited pollen flow and, consequently, to a limited gene flow between these populations.

Finally, the mixed reproductive system of *B. australis* is another life-history feature that might strengthen the genetic structure of its populations. Self-compatibility may enhance inbreeding rates that, together with genetic drift, can lead to increased genetic divergence even between close populations (Young *et al.* 1996; Dick *et al.* 2008). Federov (1966) argued that this mechanism could promote speciation even in restricted spatial scales.

In the Serra do Mar mountain range, we found a marked genetic structure on a scale as small as 6 km. Even though *B. australis* is continuously distributed along the elevation gradient, we observed no gene flow between the lowland and upland populations, isolating these extreme groups. This creates a scenario for potential adaptation to the different conditions imposed by the elevation gradient. Behind such segregation, we have not found an isolation-by-distance pattern. Elevation difference was a relevant factor though, but we need further sampling effort to check its correlation with genetic distance.

Between-altitude genetic diversity

There is a significant variation in the distribution patterns of genetic diversity within populations along altitudinal gradients (Ohsawa & Ide 2008). Other studies have shown peaks of diversity in higher (e.g. Gämperle & Schneller 2002), lower (e.g. Quiroga & Premoli 2007), and mid-elevations (e.g. Oyama *et al.* 1993; Byars *et al.* 2009). In addition, the lack of an altitudinal effect on genetic diversity is also frequently reported (e.g. Truong *et al.* 2007)

and usually denotes an unrestricted gene flow among populations along the gradient. This does not seem to be the case for *B. australis*, which has a limited gene flow among the populations from the altitudinal extremes of the Serra do Mar. Still, we did not find higher genetic diversity in the upland populations, as would be expected, considering the optimum of environmental conditions for this species. On the contrary, the lowland populations exhibited greater allelic richness and higher exclusive and rare allele counts.

A possible explanation might be the existence of forces reducing upper mountain genetic diversity, as it would be the case for bottlenecks during forest movements (Ohsawa & Ide 2008). Quiroga & Premoli (2007) suggested that *Podocarpus parlatorei* genetic decline towards higher elevations reflected forest migration during glacial periods. In the same way, Ohsawa *et al.* (2011) found that historical factors, rather than ecological ones, have primarily shaped intra-population genetic diversity distributions in montane species. The Serra do Mar mountain range formation began 65 million years ago (Hackspacher *et al.* 2004), and during this period forest ranges expanded and contracted several times following climatic oscillations. The Brazilian Atlantic montane forests that we know today emerged only 17,000 years ago (Behling 2008), and it is possible that since then some species originally from the lowland have started to climb the mountains. However, we did not find any evidence for a historical bottleneck in any population.

We could say that both populations might be already adapted to their local conditions and each has its own set of optimum conditions, both experiencing high genetic diversity levels. However, the upland site might be more selective and the survival of individuals carrying new mutations would be compromised, keeping only alleles of high adaptive value. This may have led to the elimination of rare alleles in the upland populations, explaining the presence of more exclusive and rare alleles in the lowland, as well as its higher allelic richness. Further controlled transplant experiments might elucidate this question.

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Data Accessibility

Individual DNA sequences are available in GenBank (KF267877-KF267886). Microsatellite genotype data are available in DRYAD.

Author Contributions

TSR and FAMS planned the study. TSR conducted the fieldwork with field assistants. TSR performed the laboratory work, supervised by MCG and APS. TSR, MMB and MCG analyzed the data. TSR wrote the paper, and all authors contributed to revisions.

Tables

Table 1. Genetic diversity for *Bathysa australis* populations using nine SSR polymorphic loci.

Site	Population	Coordinates	Elevation range (m)	N sampled	N total	*A*	A_e	A_R	*Ho*	*He*	*F*
Lowland	**L1**	23°22'51.23"S 45°4'45.23"W	80-120	74	827	10.44 (1.42)	4.59 (0.65)	7.60 (2.4)	0.56 (0.08)	0.73 (0.05)	0.221 (0.10)
	L2	23°22'25.81"S 45°4'50.56"W	176-198	28	107	8.11 (0.81)	4.03 (0.47)	7.02 (1.9)	0.55 (0.09)	0.72 (0.04)	0.225 (0.12)
	L3	23°22'24.70"S 45°4'55.03"W	200-216	38	110	8.78 (1.36)	4.51 (0.73)	6.89 (2.7)	0.60 (0.08)	0.72 (0.04)	0.176 (0.10)
Upland	**U1**	23°19'31.83"S 45°4'4.64"W	1050-1100	29	197	7.44 (1.02)	3.20 (0.42)	6.45 (2.4)	0.53 (0.08)	0.64 (0.05)	0.198 (0.10)
	U2	23°19'31.59"S 45°4'9.89"W	1010-1040	73	366	9.22 (1.12)	4.22 (0.49)	6.87 (2.1)	0.60 (0.06)	0.72 (0.05)	0.186 (0.04)
	U3	23°19'36.02"S 45°4'32.25"W	1010-1040	27	144	7.11 (0.82)	4.12 (0.70)	6.45 (2.2)	0.62 (0.06)	0.70 (0.05)	0.103 (0.07)
Lowland								7.171	0.573	0.738	0.224
Upland								6.591	0.588	0.707	0.169

Note: N sampled = number of individuals sampled; N total = total number of individuals in the 1-ha population plot; *A* = mean number of alleles (standard error); A_e = effective mean number of alleles (standard error); A_R = mean allelic richness (standard deviation); H_o = mean observed heterozygosity; H_e = mean expected heterozygosity; *F* = mean fixation index (standard error). Mean allelic richness across lowland populations was higher than mean allelic richness across upland populations (10,000 permutations; $P = 0.034$).

Table 2. Analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) for *Bathysa australis* populations.

Source of variation	Degrees of freedom	Sum of squares	Estimated variance	Percentage of variance (%)	*P*
Among sites (elevation band)	1	224.372	0.776	18.6	<0.001
Among populations (1-ha plots)	4	50.693	0.117	2.8	<0.001
Within populations (1-ha plots)	532	1746.016	3.282	78.6	<0.001
Total	537	2021.082	4.175		

Table 3. Pairwise F_{ST} values between *Bathysa australis* populations in the upper triangle and geographic distance (km) in the lower triangle. All F_{ST} values are significant at $P < 0.01$ (999 simulations).

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	0.048	0.049	0.242	0.206	0.209
L2	0.8	-	0.033	0.238	0.204	0.204
L3	0.9	0.1	-	0.240	0.203	0.204
U1	6.4	5.5	5.5	-	0.026	0.022
U2	6.3	5.5	5.5	0.2	-	0.014
U3	6.0	5.3	5.2	0.8	0.7	-

Table 4. Exclusive and rare allele counts across lowland and upland *Bathysa australis* populations.

Loci	Exclusive alleles		Rare alleles	
	Lowland (N = 140)	Upland (N = 129)	Lowland (N = 140)	Upland (N = 129)
BA02	3	0	4	3
BA14	8	3	7	1
BA15	9	9	10	9
BA22	2	4	3	3
BA24	4	2	5	4
BA25	3	4	14	13
BA26	3	5	9	8
BA28	5	0	5	3
BA30	20	10	18	7
Total	57	37	75[a]	51[b]

Note: different letters denote significant differences between lowland and upland populations (Wilcoxon paired test; $\alpha = 0.05$).

Figures

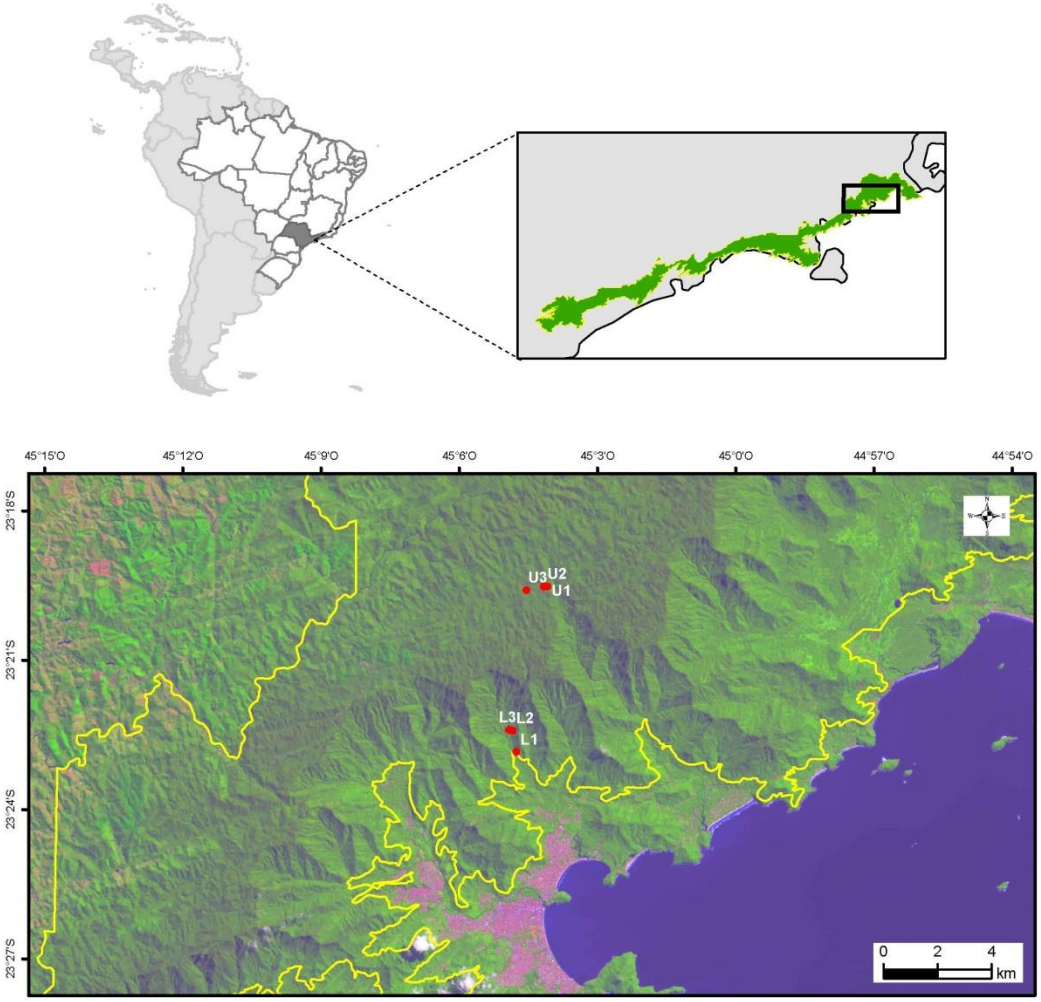

Figure 1. Map of the *Bathysa australis* populations sampled in the Serra do Mar mountain range. Location of L1, L2, and L3 lowland plots (Ubatuba, SP, Brazil), and U1, U2 and U3 upland plots (São Luis do Paraitinga, SP, Brazil), all within the limits of Serra do Mar State Park (yellow line). Image by Landsat 8 (654 RGB composition) taken in April 2014. Datum: WGS84 and 30 meters resolution.

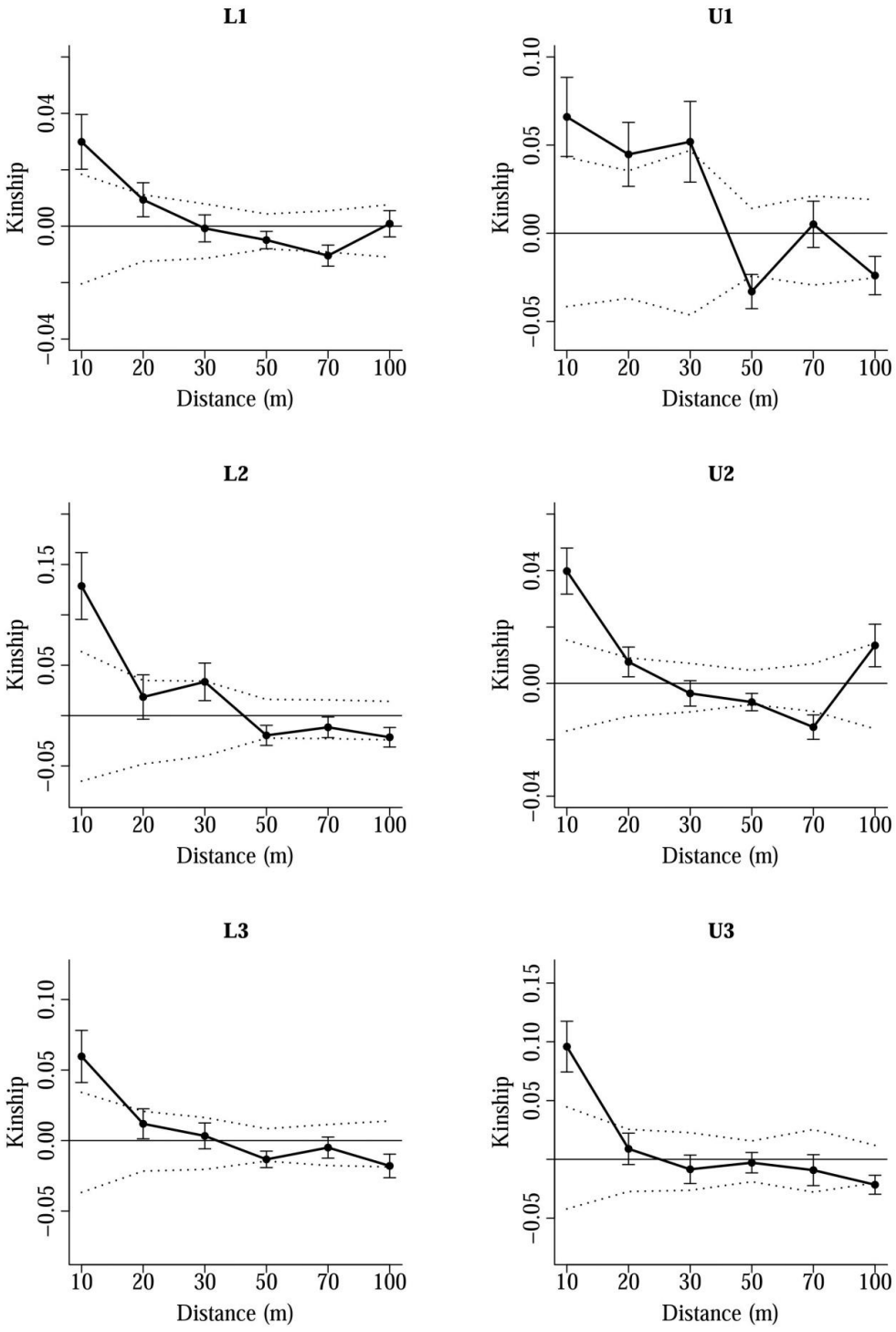

Figure 2. Fine-scale genetic structure for the six *Bathysa australis* populations. Average kinship coefficient as a function of geographical distance for each population analyzed. Bars indicate the standard deviation and dashed lines indicate the 95% confidence interval for the null hypothesis of no spatial genetic structure. Both lowland and upland populations exhibited autocorrelation in the first distance class.

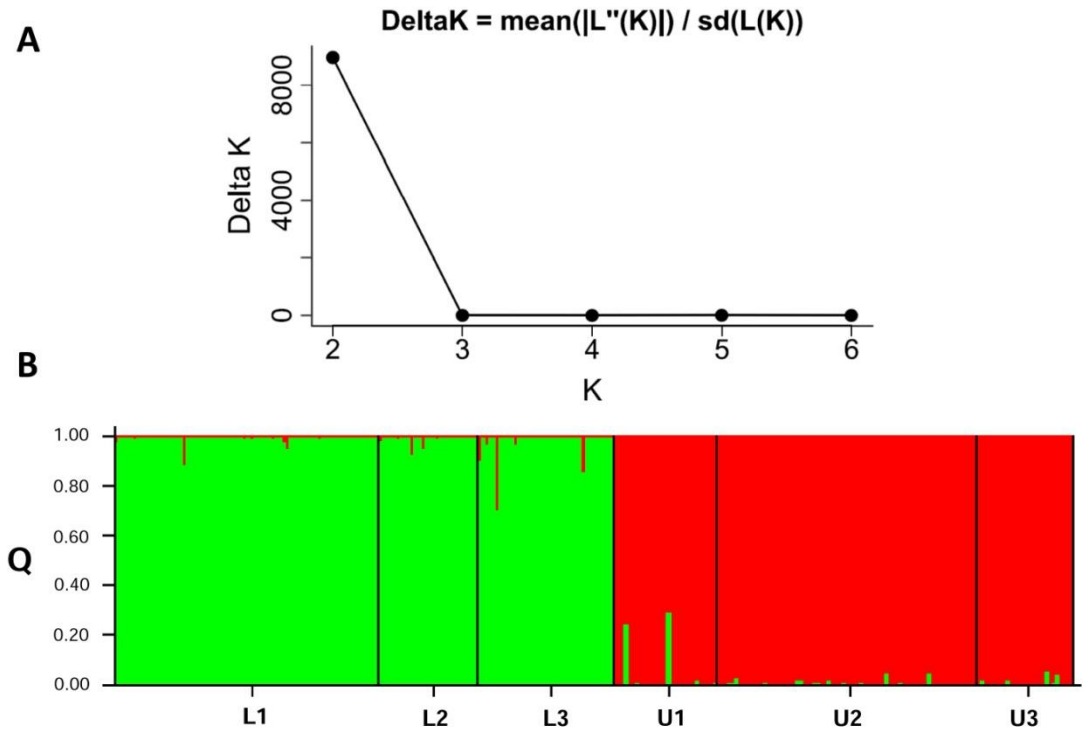

Figure 3. Genetic structure of *Bathysa australis* populations in the Serra do Mar mountain range. **A**) Graphical plot based on delta-*K* calculated according to Evanno *et al.* (2005) to estimate the actual number of clusters for the 271 *Bathysa australis* individuals used in this study. **B)** Assignment of 271 *Bathysa australis* individuals from six populations into two ($K = 2$) clusters using a Bayesian-based population genetic structure analysis carried out with the software STRUCTURE (Pritchard *et al.* 2000). Each solid bar represents a single individual, while colored areas correspond to distinct genetic clusters. Bars with multiple colors denote admixed genomes.

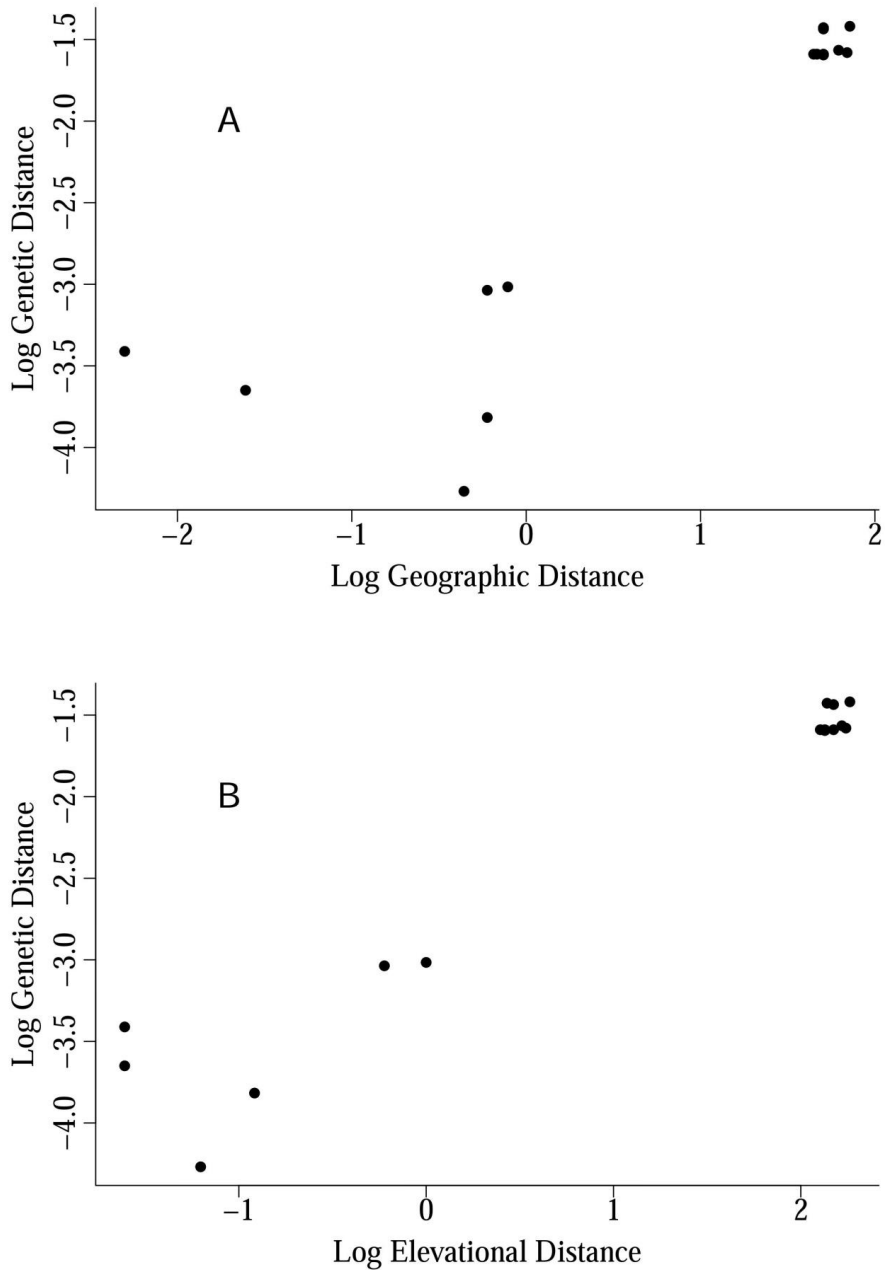

Figure 4. Isolation by distance and isolation by elevation patterns in *Bathysa australis*. Mantel correlations indicating population differentiation (pairwise F_{ST}) as a function of: **A)** geographic distance (r = 0.98, mantel-*P* = 0.02) and **B)** elevational distance (r = 0.99, mantel-*P* = 0.02). However, when the partial Mantel test discounted the effect of altitude, the pattern of isolation by distance disappeared (r = -0.38, mantel-*P* = 0.95). The pattern of isolation by elevation, on the other hand, proved consistent even after discounting the effect of geographic distance (r = 0.68, mantel-*P* = 0.02).

CAPÍTULO 3

Demography and elevation: the case of a widespread Atlantic rain forest tree species (*Bathysa australis*) in the Serra do Mar mountain range, SE Brazil

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Abstract: Elevation gradients represent steep environmental gradients over short geographic distances, being considered as natural laboratories for the study of plant responses to varying ecological conditions, a major effort to forecast population responses to environmental changes. *Bathysa australis* (Rubiaceae) is a widespread tree along the altitudinal gradient of the Serra do Mar mountain range, SE Brazil, whose upper (1010-1100 m) and lower (80-216 m.a.s.l.) populations of the gradient were chosen as our model to evaluate the influence of elevation on trait variation (size and architecture) and population fitness (vital rates and population growth, λ). Observing the reduced *B. australis* genetic diversity at the upland, and the lack of dispersal amog this and the lowland population reported in previous work, we aimed to evaluate if these elevation outcomes reflected on fitness as well. In addition, we intended to see if the past selective logging that occurred at specific plots in lowland and upland populations would interact with elevation intensifying or weakening the effects. We found that *B. australis* presented a significant demographic plasticity at this elevation gradient, as it displayed a different strategy for each altitude and both strategies successfully keep population growth ($\lambda > 1$). The slower dynamics observed at the upland ($\lambda = 1.022$ [1.011, 1.030]) was replaced by fast dynamics with higher individual growth and recruitment rates at the lowland ($\lambda = 1.084$ [1.040, 1.101]), a result consistent with the intrapopulational genetic diversity patterns. Even that upland population has shown increased flowering, this was not translated into more recruits, since lowland population had the higher recruitment. Besides increased growth, lowland plants invested relatively more in diameter than upland plants, and the height distributions were similar among altitudes. Density results pointed for greater upland abundance, compensating for lowland diameter growth and balancing total basal area among altitudes. Survival probabilities were equally high at both altitudes, a pattern that is probably related to *B. australis* resprouting capacity. This feature allowed for higher percentages of multi-stemmed individuals, which was even higher at the upland population, more prone to small-scale disturbances. Logged plots at both altitudes presented even higher multi-stem percentages than their unlogged counterparts. However, only the lowland population presented truly significant reduction in performance among logged and non-logged plots. Perhaps, the ten more years of forest recovery at the upland might explain such differences in logging impacts among altitudes. Our results have shown that a widely distributed species can perpetuate itself in a wide range of environmental conditions such as the climatic extremes along an elevation gradient, suggesting a less pessimistic view of future scenarios of climate change, at least with respect to widespread species with demographic plasticity.

Key-words: Altitude, fitness, Integral Projection Model, LTRE, population growth rate, selective logging.

Introduction

In ecology, much attention has been given to the influence of environmental gradients in populations and plant communities (Gentry 1988, Givnish 1999). Abiotic gradients represent natural laboratories for the study of plant responses to varying environmental conditions (Malhi et al. 2010, Picó 2012, De Frenne et al. 2013). Investigations on how species' cope with environmental heterogeneity along its range are necessary to understand the relationship between environment and fitness, an important initial effort to forecast population responses to environmental changes (Angert 2009). In addition, the study of within-species trait variation in geographic space provides us with insights on the selection pressures driving population divergence and habitat preferences (Guo et al. 2010). In this sense, species with wide ranges are perfect models to evaluate such trait (e.g. size) and fitness (e.g. vital rates) variation considering the large amplitude of environmental conditions they experience.

Elevation gradients, for instance, are special cases of landscape variation that represent steep environmental gradients over short geographic distances, which has major influence on population fitness, particularly for those populations located marginally at the upper and lower distribution limits (Herrera and Bazaga 2008, e.g. Byars et al. 2009). At this kind of gradient, factors such as temperature, precipitation, and soil characteristics vary sharply (Grubb and Whitmore 1966, Grubb 1977, Gentry 1988, Lieberman et al. 1996), and this variation is in part due to the occurrence of fog at higher elevations, which increases atmospheric moisture and reduces solar radiation (Pendry and Proctor 1996, e.g. Bruijnzeel et al. 1993, Wang et al. 2007). These combined changes along the gradient are expected to affect habitat suitability for a species and there is a vast literature evaluating how altitudinal gradients influence the many features involved in a plant's life cycle (e.g. Eriksen et al.1993, Hemborg and Karlsson 1998, Bühler and Schmid 2001, Fabbro and Körner 2004, Fernández-Calvo and Obeso 2004, Cierjacks et al. 2008, Brito and Sazima 2012).

The decrease in individual growth with increasing elevation has been frequently reported (Grant and Mitton 1979, Fernández-Calvo and Obeso 2004, Coomes and Allen 2007, King et al. 2013), and might be compensated by higher efforts on reproduction (Hemborg and Karlsson 1998, Fabbro and Körner 2004, Brito and Sazima 2012). In a set of alpine plants from the Swiss Alps, for example, reproduction was prioritized over growth by means of

prolonged flowering and higher biomass allocation to reproductive structures (Fabbro and Körner 2004). In the same way, Brito and Sazima (2012) found denser flowering at the high-altitude populations of a shrub species in the Serra do Mar mountain range in southeast Brazil. Nevertheless, fruit and seed set were constrained by the low pollinator abundance at this same site, which corroborated previous findings in artic systems (Eriksen et al. 1993, Hemborg and Karlsson 1998). This somewhat low regeneration of plant populations was also reported for other high elevation sites (Bühler and Schmid 2001, Cierjacks et al. 2008), and the same limitations to the vegetative growth have been suggested to also restrict reproduction, reducing seedling numbers (Cierjacks et al. 2008).

However, the opposite trend has also been observed, as low seed production coupled with reduced seedling survival limited the recruitment of a Mediterranean species in the southernmost margin of its altitudinal range (Giménez-Benavides et al. 2008). In this case, the overall pattern of low population fitness on high-elevation sites was substituted for improved fitness in a typical high mountain plant from the Mediterranean basin. It draws attention to the role of species-specific optimal environmental conditions, that is, the 'place' in an environmental gradient where the species find the most of its ecological needs among a series of niche axis (Brown 1984, Eckert et al 2008).

Plant traits are also known to vary with altitude (Gugerli 1997, Fabbro and Körner 2004, Guo et al. 2010). Those traits related with plant size and architecture are generally affected towards more frequent multi-stemmed and small stature plants with increasing altitude (e.g. Fernández-Calvo and Obeso 2004, Bellingham and Sparrow 2009, Fisher et al. 2013, Asner et al. 2014). Upland sites are usually at the highest slopes where terrain is more unstable (Poorter et al. 1994), and exposed to wind blow, therefore subject to frequent small-scale disturbances that favor the occurrence of multi-stemmed individuals through higher damage and sprouting. In addition, the lower productivity of upland sites (Bruijnzeel & Veneklaas 1998, Girardin et al. 2010) usually means restricted individual growth (e.g. Coomes and Allen 2007), and in high productive sites, where height growth is unrestricted, the multi-stemmed architecture is not advantageous since it promotes more shading (Bellingham and Sparrow 2009). However, when a productive lowland forest is subject to frequent disturbances, then multi-stemmed architecture might be advantageous. In this sense, both natural and anthropogenic disturbances, even if as low impact activities (e.g. selective logging), might have major consequences for forest structure and dynamics (e.g. Villela et al. 2006), weakening or enhancing the elevation effects.

The balance of it is a wide variation in plant community structure, composition (Grubb 1977; Gentry 1988; Lieberman et al 1996; Oliveira-Filho and Fontes 2000, Sanchez et al. 2013; Toledo-Garibaldi and Williams-Linera 2014), and species interactions (Cruden 1972, Eriksen et al. 1993, Fernández-Calvo and Obeso 2004, Hillyer & Silman 2010, Brito and Sazima 2012) along altitudinal gradients, which again affects species' performance. Likewise, in the Atlantic rain forest of the Serra do Mar mountain range (SE Brazil), an altitude increase resulted in differences in the plant community structure, composition, species richness and also in the size of plants (Oliveira-Filho and Fontes 2000, Alves et al. 2010, Joly et al. 2012, Scaranello et al. 2012, Eisenlohr et al. 2013, Lacerda et al. 2013). In this area, according to the Brazilian National Classification System for Vegetation (IBGE System; Veloso et al. 1991), two types of vegetation can be easily found because of the vast area they occupy, a Submontane vegetation (50 to 500 m a. s. l.), hereafter called lowland, and a Montane vegetation (500 to 1200 m), hereafter called upland. Populations of species at this different vegetation types along the elevation gradient in the Serra do Mar mountain range would be subject to very distinct environmental conditions, as fog formation, and decreases in soil temperature and precipitation have been reported at the upland (Alves et al. 2010, Souza Neto et al. 2011, Eisenlohr et al. 2012, Joly et al. 2012).

Bathysa australis (A. St.-Hil.) Hook. f. ex K. Schum. (Rubiaceae) is a tropical tree geographically endemic to the Brazilian Atlantic forest, but widely distributed along the altitudinal gradient of the Serra do Mar. For this reason, *B. australis* populations at the upper and lower parts of the gradient were chosen as our model to evaluate the influence of elevation on trait variation (size and architecture) and population fitness (vital rates and population growth, λ). Considering that lowland and upland populations of this species are experiencing very distinct environmental situations, we expect such differences to reflect both on trait pattern and performance. Moreover, taking into account that reductions on *B. australis* genetic diversity has already been reported for the upland population (Reis et al. 2015), and assuming that the amount of genetic diversity found in a population is expected to be correlated with its current fitness (Reed & Frankham 2003), we aimed to evaluate if these elevation outcomes on genetics reflected on fitness as well. Besides, previous work has also found a lack of gene flow among lowland and upland populations (Reis et al. 2015), indicating these sites are not connected through dispersal, thereby isolating this factor and suggesting that only local factors are responsible for the demographic patterns of this species at each site.

In addition to the altitude effect, *B. australis* populations in the Serra do Mar mountain range might be influenced by the past selective logging that occurred at specific areas in both lowland and upland sites. The logging activity did not include *B. australis* populations directly, since only species of high wood value were target. At the upland, one of the population plots was subject to selective logging until 1970 (Padgurschi et al. 2011), while at the lowland another plot was explored until 1982 (Ramos et al. 2011). Although it represents a low impact activity compared to forest clearcut, the habitat transformation through continuous removal of selected trees might have significant consequences for populations' structure and dynamics (e.g. Villela et al. 2006), even after 30-40 years of forest recovery. This will probably be the case of *B. australis*, a species whose seedlings present rapid biomass responses to the varying light environments, and seeds depend on light for germination (Duz et al. 2004).

In this study, we aimed to investigate primarily how elevation affects the structure and demography of *B. australis* populations at two extreme sites in the altitudinal gradient of the Serra do Mar mountain range, southeast Brazil. Our expectation was to find reduced fitness, and biomass at the upland site, compared to the lowland, if this habitat truly offers harsher conditions for *B. australis* growth, survival and reproduction, as suggested by genetic diversity results. We also expect architectural changes towards relative smaller sizes and more frequent multi-stemmed individuals at the high-altitude site, as the outcome of presumed lower productivity, due to the observed lower temperatures at this upland site (see Souza Neto et al. 2011), and frequent small-scale disturbances. Specifically, *B. australis* size (maximum height), density, biomass, height growth, survival, fecundity, and λ would be reduced at the upland compared to the lowland (negative elevation effect), while the multi-stemmed frequency would be enhanced (positive elevation effect).

Additionally, we believe that the historical of logging, primarily through higher frequency of small-scale disturbances, but also through increased light availability in a lesser extent, will interact with altitude affecting our results by intensifying or weakening the expected changes in the upland and lowland sites. At last, if we find a variation among *B. australis* population plots that is neither explained by altitude or logging, then we have evidence of a locally varying structure and demography mostly attributed to the environmental space, at axis not evaluated in this study, and whose importance is greater than we expected.

Materials and Methods

Study species

B. australis is a tree species of the Rubiaceae family that belongs to the lower strata of the forest canopy, reaching up to 20 meters in height. It is an endemic species of the threatened Atlantic rain forest, one of the Brazilian biodiversity hotspots (Meyers et al. 2000). This species is distributed predominantly in South and Southeast Brazil (Germano-Filho 1999), existing as common plant in parts of the Atlantic forest (e.g. Ramos et al. 2011), where it has an important role in ecosystem functioning, e.g., providing a nectar source to a variety of insects (Freitas and Andrich 2013). Furthermore, its bark is used in folk medicine (Germano-Filho, 1999), which indicates its social value in addition to its ecological value.

Reproduction occurs annualy and the flowering period is usually from December to April, while fructification is from February to June (Freitas and Andrich 2013). *Bathysa* displays terminal thyrsus inflorescences and its flowers are hermaphrodite, homostylous, and self-compatible (Freitas and Andrich 2013). In spite of its compatibility system, *Bathysa* flowers are dichogamous, a temporal separation of the male and female functions that may promote outcrossing in hermaphrodite flowers (Freitas and Andrich 2013). The key pollinators are social bees and wasps (Freitas and Andrich 2013). The fruit is a capsule of 4-6 mm in length, and seed dispersal is autochoric (Ziparro et al. 2005; Colloneti et al. 2009).

Other important biological features of *B. australis* include dependence on light for germination and great plasticity in the use of the light environment during its initial development (see Duz et al. 2004). *B. australis* can take advantage of high light availability opportunities for growth and recruitment, but even at shading conditions *B. australis* seedlings are capable of improving light absorption by increasing the leaf area ratio and decreasing root/shoot ratio, keeping positive relative growth rates (Duz et al. 2004).

Study site and sampling design

This study was conducted during 2010-2012 at two sites along the elevation gradient (80–1100 m a.s.l.) of the Atlantic forest of the Serra do Mar mountain range in São Paulo State, SE Brazil (Figure 1). Upland (1010-1100 m) and lowland (80-216 m) sites are within the Serra do Mar State Park in the municipalities of São Luís do Paraitinga and Ubatuba, respectively. At both sites, *B. australis* populations were sampled in 1-ha plots, already established for the project “Biota Gradiente Funcional” (see Joly et al. 2012). In total, six 1-ha plots were sampled: three at the lowland site (L1, L2, and L3) and three at the upland site (U1,

U2, and U3). Distances among plots within each site varied between 100 - 900 m, and distance among sites was approximately 6 km.

The two sampled sites differ in climate and precipitation. Whilst the lowland regional climate is tropical humid, the upland climate is subtropical humid, both with no dry season (Setzer 1966). The lowland mean annual temperature is 22 °C and the average annual rainfall exceeds 2,500 mm. Even in the driest months, from June to August, the average monthly precipitation is above 60 mm. The upland average annual temperature is 20 °C and the average annual rainfall exceeds 1,100 mm. In the driest months, from April to September, the average monthly precipitation is above 30 mm (EMBRAPA 2003).

According to the Brazilian National Classification System for Vegetation (IBGE System; Veloso et al. 1991), the upland sites are covered by Montane Dense Ombrophilous Forest and the lowland sites are covered by Submontane Dense Ombrophilous Forest. Indeed, these two sites differ markedly in vegetation structure, composition (Alves et al. 2010, Joly et al. 2012, Eisenhlor et al. 2013), soil temperature (Souza Neto et al. 2011), but not in soil type (Martins 2010).

Both sites have an historical of selective logging for timber products at specific areas with reported differences on community structure, diversity, and composition (e.g. Padgurschi et al. 2011, Ramos et al. 2011). At the upland site, the U3 plot was subject to logging until 1970 (Padgurschi et al. 2011), and at the lowland site, the L1 plot was explored until 1982 (Ramos et al. 2011).

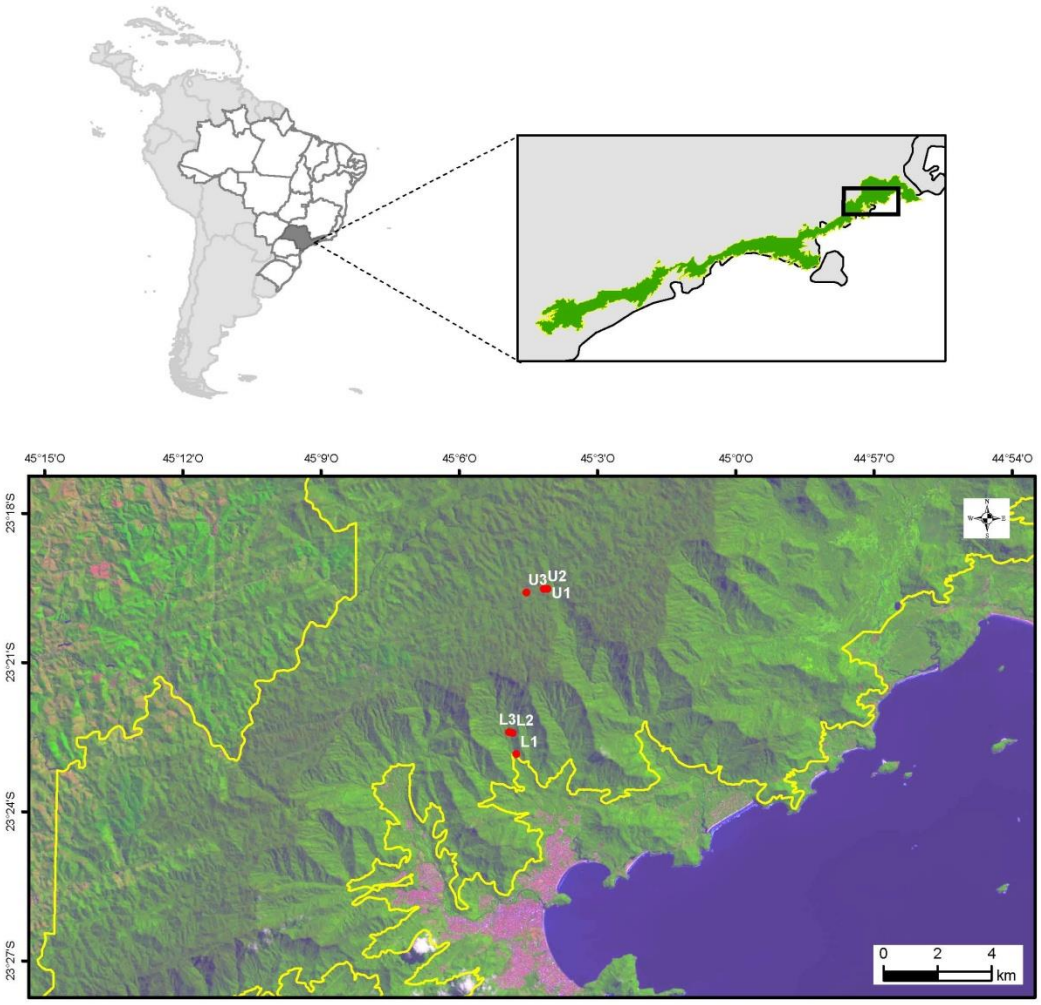

Figure 1. Map of the Serra do Mar mountain range indicating the location of the six *B. australis* population plots. L1, L2, and L3 lowland plots (Ubatuba, SP, Brazil), and U1, U2 and U3 upland plots (São Luis do Paraitinga, SP, Brazil) are all within the limits of Serra do Mar State Park (yellow line). Image by Landsat 8 (654 RGB composition) taken in April 2014. Datum: WGS84 and 30 meters resolution.

Field measurements

For practical purposes, each 1-ha plot was divided into 100 subplots of 10 x 10 m. Between February and April 2010 every *B. australis* individual with leafs or cotyledons that could be recognized at the field was marked with a tag, mapped and measured. Measurements consisted of maximum stem height and basal diameter for all individuals. Small plants were measured for stem height with an ordinary ruler and bigger plants with a 15 meters dendrometric ruler. The few plants that exceeded 15 meters had their height estimated. Those with more than one stem - hereafter called multi-stemmed individuals - had their basal

diameter measured for each stem. We also verified the reproductive status of adult individuals searching for reproductive structures. Subsequent annual censuses (2011 and 2012) were also made during the flowering period, between February and April. We took the same measurements for all surviving individuals, and those not found at the precise coordinate were considered dead.

Recruits

Newly sampled individuals each year (2011 and 2012) were also measured for maximum height and basal diameter. However, these were not considered recruits at first, since the plot area was too large and some of them could have been missed from earlier census. For better assign the recruits we adjusted linear or quadratic functions based on mean growth of the correctly measured individuals in all census year to estimate the previous measures of all newly sampled individuals. We grouped individuals into two size classes - small (< 1 m height) and large (> 1 m height) - for the accurate estimation of growth in each class, as they represented different growth behaviors. A model was then constructed for each size category, population plot, and transition year (Appendix A). Those individuals with previous estimated size measures lower than 0.04 m were considered recruits, and those with 0.04 m or bigger, were considered 'missed individuals'. In the latter case, we used these estimated size measures for all the analyses, including vital rates calculation, with the exception of growth.

Population density, basal area, and size structure

Density and total basal area estimates per 1-ha plots were provided for each plot and site (pooled unlogged plots). These estimates were calculated for each census year and had 95% confidence intervals estimated through bootstrapping. Basal area bootstrapping consisted of resampling individuals with replacement from the datasets and recalculating the statistics (N = 1,000 trials). For the density bootstrapp we used the 100 subplots of 10 x 10 m for individual counting. Comparisons amog sites and plots were performed with randomization tests though. By permuting labels we reshuffled individuals (basal area) and subplots (density) among samples before recalculating statistics. This was repeated 1,000 times and P-values were obtained through the mean of all absolute simulated differences greater than the absolute observed difference among statistics.

Size distributions were compared between plots each year using maximum stem height measures and the Kolmogorov-Smirnov test with a Bonferroni correction for multiple

comparisons. Hereafter, every time we mention size we are referring to the maximum stem height measures since this variable was considered the one that best represents *B. australis* state through its life cycle. Stem height was chosen over diameter because *B. australis* irregularities at the stem base could provide biased basal area estimates, and also because of stem height ecological relevance, since it informs us about the plant vertical position on the forest light profile, which determines the plant access to light (Paine et al. 2015).

Multi-stemmed individuals

To verify if lowland and upland sites differed in the number of multi-stemmed individuals, we quantified the exact number of stems per individual, and the total number of single- and multi-stemmed individuals in each plot and year. In both cases we performed Chi-square tests for each year. We also compared plots and sites (pooled unlogged plots) for the percentage of multi-stemmed individuals constructing confidence intervals after 1,000 bootstraps and performing randomization tests. Bootstrapping consisted of resampling individuals with replacement from each sample dataset and recalculating the statistics (N = 1,000), while randomizations consisted of permuting labels thereby reshuffling individuals among samples before recalculating statistics. Randomizations were repeated 1,000 times and P-values were obtained through the mean of all absolute simulated differences greater than the absolute observed difference among statistics. Since we did not find differences among years for each plot, we pooled all year's data.

Vital rates among sites and plots

We tested for differences in vital rates among sites and plots with Generalized Linear Models (GLMs). We build a model with the whole dataset in order to evaluate the effect of site on each vital rate, and we build models for each lowland and upland site to evaluate if there was a significant variation among plots within sites. Logistic models (with binomial error distribution and logit link function) were applied for survival probability (P_{surv}) and flowering probability (P_{flower}). Individual growth (μ), on the other hand, was described by applying linear models because it represented the relationship among *Size* and *Size Next*, which usually has a linear behavior (e.g. Li et al 2013). We tested for the main effects of *Size*, *Site* and *Plot*, and for the interactions among *Size* × *Site,* and *Size* × *Plot*.

Mortality and recruitment rates

We calculated annual mortality and recruitment rates for each plot and site (pooled unlogged plots) with 95% confidence intervals estimated through bootstrapping, which consisted of resampling individuals with replacement from each sample dataset and recalculating the statistics (N = 1,000). We tested for differences amog samples with randomization tests, that is, permuting labels thereby reshuffling individuals among samples before recalculating statistics. This was repeated 1,000 times and *P*-values were obtained through the mean of all absolute simulated differences greater than the absolute observed difference among rates.

Annual mortality rates were calculated as: $m = 1- (N_t/N_0)^{1/t}$, where N_t is the number of surviving plants at the end of the interval, N_0 is the number of plants at the beginning of the interval, and t is the time interval in years (Sheil et al. 1995). Annual recruitment rates were calculated as: $r = 1 – (1- nr/N_t)^{1/t}$, where N_t is the number of surviving plants at the end of the interval, nr is the number of recruits in the interval, and t is the time interval in years (Sheil et al. 2000).

Demographic models

To assess the effects of altitude on *B. australis* population dynamics, we constructed integral projection models (IPMs) using code from the R library IPMpack (Metcalf et al. 2013). Unlike matrix models, IPMs describe population dynamics using a continuous individual-level state-variable (e.g. maximum height) instead of a set of discrete-stages (Easterling et al. 2000). Populations structured by this state-variable have their changes tracked in discrete time trough functions that describe size-dependent growth, survival and fecundity (Merow et al. 2014). Relationships between the state-variable and these vital rates were established by fitting constant, linear, and quadratic functions to the data, and selecting the best fit based on lowest Akaike Information Criteria (AIC). The selected functions together make up the kernel function that links the size x distribution of individuals at time t, $n(x, t)$, to their size y distribution $n(y, t + 1)$ in the next studied period, $t + 1$ (Easterling et al. 2000). So, the integral projection model for the number of individuals of size y at time $t + 1$ is:

$$n(y,t+1) = \int_{L}^{U} [p(x,y) + f(x,y)]n(x,t)\,dx$$

$$= \int_{L}^{U} k(y,x)n(x,t)dx$$

where [L,U] is the range of all possible sizes x or y, $p(x,y)$ represents survival and growth from size x to size y, and $f(x, y)$ represents the number of recruits of size y produced by parents of size x. $p(x,y)$ was calculated as $p(x,y) = s(x)\ g(y, x)$, where $s(x)$ is survival and $g(y, x)$ is a normal probability density function with mean future plant size ($\mu(x)$) and growth variance ($\sigma^2(x)$). To avoid eviction problems (Williams 2012), that is, individuals with predicted future size outside the range limits, we set each model with a minimum size 10 % less than the minimum observed sizes (observed minimum: 0.03 m at lowland, and 0.02 m at upland) and a maximum size 10 % higher than the maximum observed size (observed maximum: 17 m at lowland, and 21 m at upland). We applied the mid-point rule to convert the kernel function into a large transition matrix with 100 mesh points (Easterling et al. 2000).

Because *B. australis* individuals produce a huge amount of fruits, we could not quantify the number of fruits (and seeds) for each individual. We therefore estimated a constant for each population representing the mean number of established seedlings produced per reproductive individual (*pe*) as $pe = n/N$, where n is the number of established seedlings in the population, and N is the number of reproductive individuals. The function describing reproduction, $f(x, y)$, was then the product of the size-dependent probability of flowering (P_{flower}), the mean number of established seedlings per reproductive individual (*pe*), and a probability function of the seedling size distributions. The seedling size distribution in each population was described by a normal distribution with the observed mean (φ_1) and standard deviation (φ_2).

We decided to pool the demographic data collected during 2010-11 and 2011-12 transitions since we observed a somewhat constancy in the structure of populations among years. This choice was also influenced by the fact that only two time transitions are not enough to build a stochastic model (see Miller et al. 2009, Bruna et al. 2014).

Although genetic structure analysis has pointed out for only two genetic clusters, each corresponding to a site (Reis et al. 2015), we decided to build six integral projection models, one for each population plot, as we observed a significant variation within sites with respect to population structure. Besides, the selective logging that occurred in population plots L1 and

U3 in the past might have affected these population dynamics. Among all plots, the U3 showed no recruitment during the studied period, and for this reason we had to use the fecundity parameters (*pe*, φ_1, and φ_2) from the pooled upland site data to supply the U3 plot model construction (Table 5).

For each IPM, we calculated the deterministic population growth rate (λ) with a 95% bootstrap confidence interval for lambda. We applied the bootstrapping method resampling individuals with replacement from the datasets. Then we recalculated regression coefficients, constructed the kernel and calculated λ. This was repeated 1,000 times, and the 95% confidence intervals for λ were obtained from the frequency distribution of these values. We also performed a randomization test in order to compare λ's among plots and sites (N = 1,000). By permuting labels we reshuffled individuals among samples before recalculating statistics.

Elasticity and LTRE analysis

We calculated the elasticities for all IPM transitions, that is a prospective demographic perturbation analyses that predicts the changes in λ that would result from any specified change in the vital rates (Caswell 2010). We also performed the retrospective analyses of Life Table Reponses Experiment (LTRE) to quantify the effects of vital rates and transitions on observed differences in population growth rates among treatments. LTRE was calculated as follows:

$$\Delta\lambda = \lambda^t - \lambda^c \approx \sum_{ij} (a_{ij}^t - a_{ij}^c) \times \left(\frac{\partial\lambda}{\partial a_{ij}}\right)\Bigg|_{(A^t+A^c)/2}$$

Where λ^t and λ^c are measures of λ from treatment and control matrices, a^t_{ij} and a^c_{ij} represent each corresponding matrix element of the treatment and control matrices, and $\partial\lambda/\partial a_{ij}$ correspond to the sensitivity of that element for the averaged across the two matrices ($A^t + A^c$)/2. Thus, we have a matrix of differences outweighed by the sensibility of the mean matrix (Caswell 2001, Bruna and Oli 2005). To evaluate the effect of altitude on *B. australis* demography we pooled all plots within sites and the main underlying drivers of the $\Delta\lambda$ between lowland and upland were accessed. In the same way, to evaluate the effects of logging we contrasted the pooled non-logged plots to the logged plot within a site and examined the main drivers of their $\Delta\lambda$. In our study, control and treatment matrices were arbitrarily defined.

All the above analyses were performed in R software (R Development Core Team 2008).

Results

Population density, basal area, and size structure

During our three years study, we surveyed 2,229 *B. australis* individuals, 1,283 at the lowland site, and 946 at the upland site. In spite of lowland site greater absolute number of individuals, this was not a consistent pattern among plots, but rather an effect of the logged plot L1, denser than any other plot ($P < 0.001$, $N = 1{,}000$ permutations for all comparisons – Appendix B1; Figure 2). At the upland site though, the variance among plots was lower, but U2 plot presented higher density than the logged U3 plot ($P < 0.01$, $N = 1{,}000$ permutations – Appendix B1; Figure 2). Excluding both logged plots from the analysis, we found a higher mean density for the upland ($P < 0.001$, $N = 1{,}000$ permutations; Figure 2).

Total basal area pattern resembled the density pattern in the sense that L1 logged plot presented again extremely higher values than any other plot ($P < 0.001$, $N = 1{,}000$ permutations for all comparisons – Appendix B2; Figure 3). All the other plots had similar total basal area estimates. This suggests that within site variation due to logging was important again for basal area at the lowland site, but that in this case variation among sites was not significant. Variance among years was small and we did not found any significant difference, neither for density or total basal area (Tables 1, and 2).

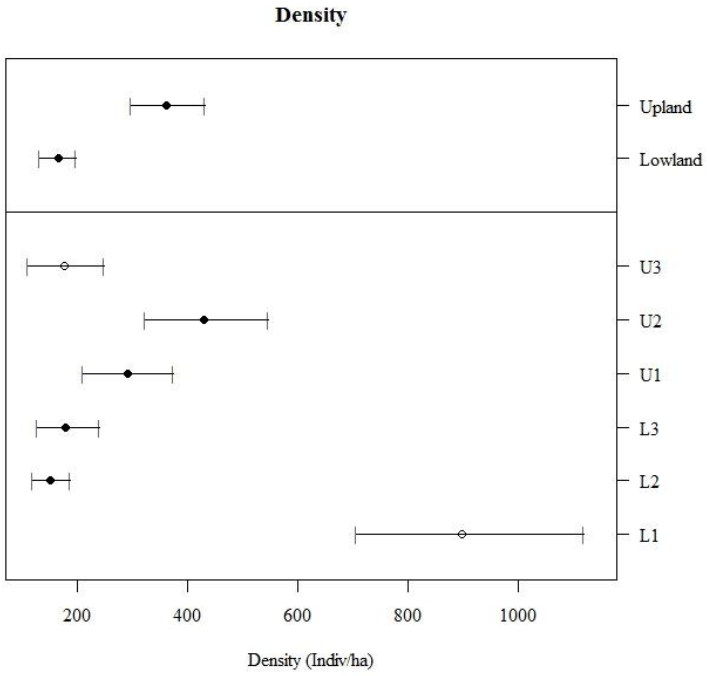

Figure 2. Number of *B. australis* individuals in 1-ha plots during 2012 census with bootstrapped 95% confidence interval generated from 1,000 bootstrap simulations (2010 and 2011 census were omitted due to redundancy, see Table 1). Pooled upland and lowland densities excluded the respective logged plots. Empty circles represent logged plots and filled circles the unlogged ones. Excluding logged plots, upland showed the higher mean density ($P < 0.001$, $N = 1{,}000$ permutations).

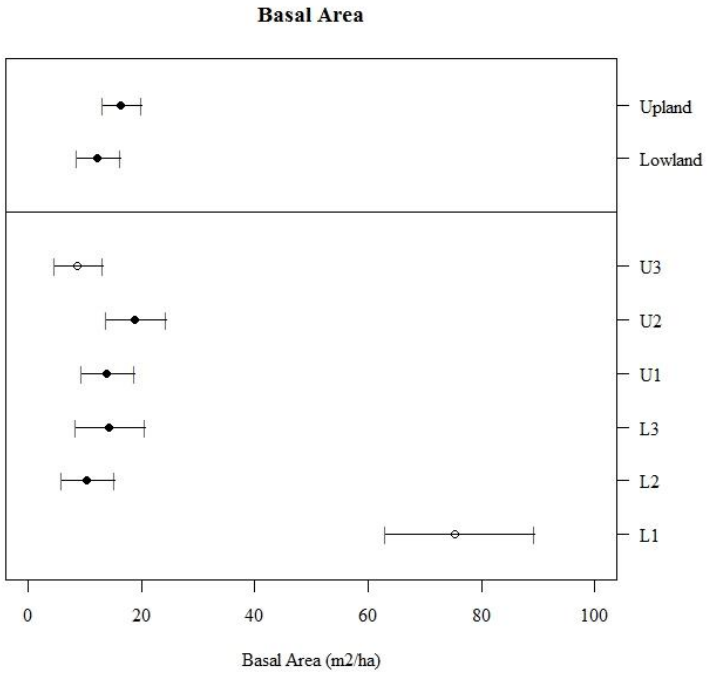

Figure 3. Total basal area estimates (m^2) in 1-ha plots during 2012 census with bootstraped 95% confidence intervals generated from 1,000 bootstrap simulations (2010 and 2011 census were omitted due to redundancy, see Table 2). Pooled upland and lowland densities excluded the respective logged plots. Empty circles represent logged plots and filled circles the unlogged ones. Excluding logged plots, upland and lowland presented similar basal area estimates ($P > 0.05$, $N = 1{,}000$ permutations).

Table 1. Density estimates (individuals.ha^{-1}) with bootstraped 95% confidence intervals between brackets for the six *B. australis* populations in each census year. * Logged plots (L1 and U3) excluded from the analysis.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**	**Lowland***	**Upland***
2010	924 [714, 1130]	144 [113, 180]	172 [122, 222]	290 [216, 375]	449 [336, 574]	188 [119, 268]	158 [130, 191.5]	369.5 [302, 447]
2011	912 [730, 1136]	155 [119, 195]	184 [136, 243]	297 [216, 388]	449 [342, 574]	180 [115, 253]	169.5 [134.5, 203.5]	373 [300, 455]
2012	898 [707, 1121]	151 [118, 187]	178 [128, 241]	291 [210, 375]	430 [323, 547]	176 [109, 249]	164.5 [132, 197.5]	360.5 [297.5, 431.5]

Table 2. Total basal area estimates (m^2.ha^{-1}) with bootstraped 95% confidence intervals between brackets for the six *B. australis* populations in each census year. * Logged plots (L1 and U3) excluded from the analysis.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**	**Lowland***	**Upland***
2010	75.2 [63.2, 88.2]	11.4 [5.5, 18.2]	13.1 [8.2, 18.8]	14.5 [9.9, 19.4]	18.2 [13.4, 23.4]	8.4 [4.5, 13.0]	12.3 [8.4, 16.5]	16.3 [13.2, 19.9]
2011	74.6 [62.5, 86.3]	10.4 [6.0, 15.9]	13.4 [8.4, 19.4]	14.7 [10.3, 20.1]	17.6 [13.0, 22.9]	8.3 [4.6, 12.9]	11.9 [8.3, 15.5]	16.1 [12.9, 19.9]
2012	75.2 [63.1, 89.4]	10.3 [5.9, 15.3]	14.3 [8.5, 20.6]	13.9 [9.6, 18.9]	18.9 [13.8, 24.4]	8.6 [4.7, 13.3]	12.3 [8.7, 16.4]	16.4 [13.2, 20.1]

The observed size distributions of all population plots were positively skewed (Figure 4). However, L1 population stood out in relation to the others because it showed relatively less small- and medium-size individuals for all census years (Table 3). All other plots were similar, although U2 plot seemed to have a higher frequency of smaller size individuals, and L3 plot seemed to have a small peak around 10 m size individuals. We also did not found size structure differences between years for any of the plots (Figure 4).

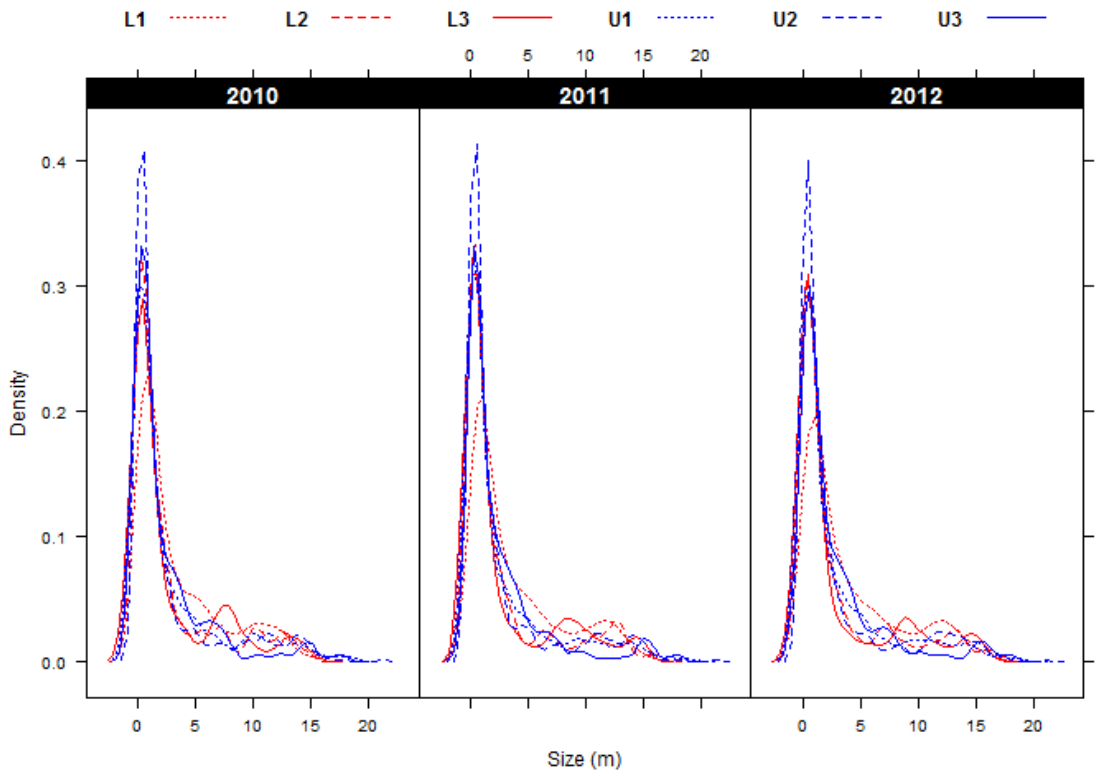

Figure 4. Size structure of *B. australis* lowland and upland population plots in the Serra do Mar mountain range in 2010, 2011, and 2012 demographic census.

Table 3. Comparisons between populations size distributions using Kolmogorov-Smirnov tests. The maximum differences between cumulative size distributions (D_{max}) are presented. Only 2012 results are shown because there was no difference between years. Population L1 had less small and medium size individuals than all other populations.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	0.298*	0.343*	0.292*	0.262*	0.233*
L2		-	0.069 NS	0.066 NS	0.077 NS	0.137 NS
L3			-	0.076 NS	0.132 NS	0.196 NS
U1				-	0.102 NS	0.161 NS
U2					-	0.081 NS
U3						-

Significance codes: NS Non-significant, * $P < 0.003$ (Bonferroni α-corrected)

Multi-stemmed individuals

The relative frequency of single- and multi-stemmed individuals was different among *B. australis* population plots in all years (2010: $\chi^2_{(5)} = 29.45$, $P < 0.0001$, 2011: $\chi^2_{(5)} = 41.92$, $P < 0.0001$, and 2012: $\chi^2_{(5)} = 44.25$, $P < 0.0001$), as was the percentage of multi-stemmed individuals for pooled years data (Figure 5). As expected, we found more multi-stemmed individuals in L1 and U3 plots (see Appendix B3 for each comparison), precisely those with a history of anthropogenic disturbance. U3 plot presented even more multi-stemmed individuals than L1 plot ($P < 0.001$, $N = 1{,}000$ permutations – Appendix B3), which is in accordance with the expectation of prevalent multi-stemmed architecture at the upland site. Excluding logged plots, upland showed the higher percentage of multi-stemmed individuals ($P < 0.001$, $N = 1{,}000$ permutations; Figure 5). However, when considering the exact number of stems per individual we found a higher frequency of two- and three-stemmed individuals only for U3 plot (2010: $\chi^2_{(15)} = 39.24$, $P < 0.001$, 2011: $\chi^2_{(15)} = 37.48$, $P < 0.01$, and 2012: $\chi^2_{(15)} = 51.0$, $P < 0.0001$; Figure 6). The annual oscillation observed for three-stemmed individuals in U3 plot was due to an increase in total population abundance in 2011 that was not immediately followed by an increase in three-stemmed individual's abundance, which occurred only in 2012.

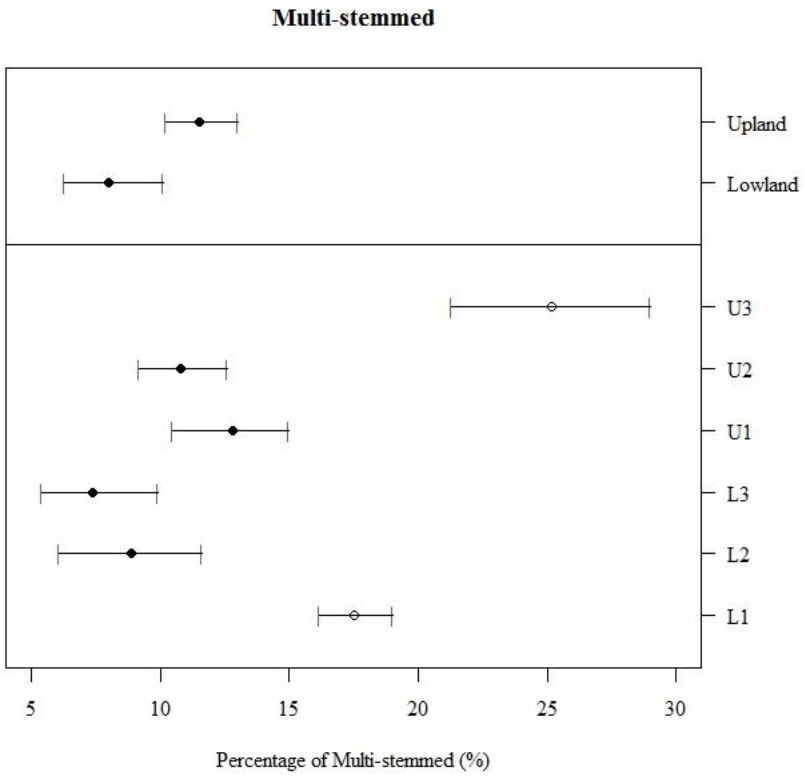

Figure 5. Percentage of multi-stemmed individuals with bootstrapped 95% confidence intervals (N = 1,000 simulations) for each lowland and upland *B. australis* population plot considering all year's pooled data. Pooled upland and lowland datasets excluded the respective logged plots. Empty circles represent logged plots and filled circles the unlogged ones. Excluding logged plots, upland showed the higher percentage of multi-stemmed individuals ($P < 0.001$, N = 1,000 permutations).

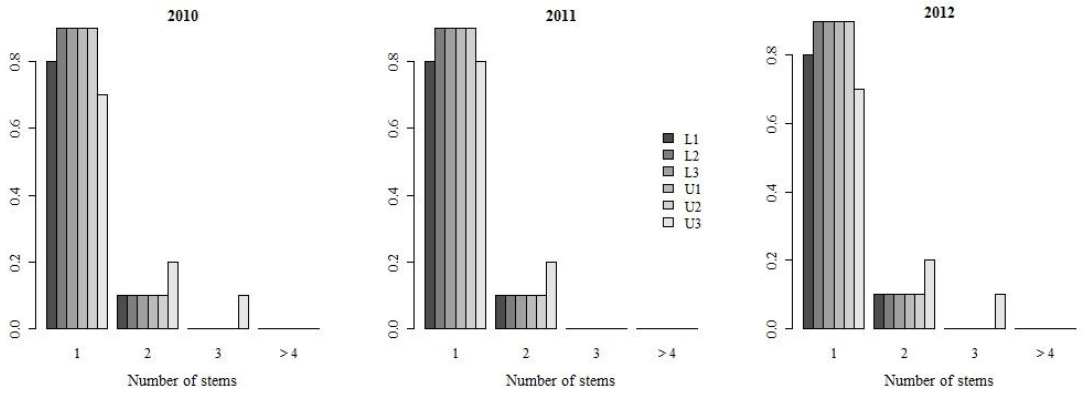

Figure 6. Individual stem number frequency for lowland and upland *B. australis* population plots in 2010, 2011, and 2012 census year. There was a higher frequency of two-, and sometimes three-stemmed individuals for the U3 plot (2010: $\chi^2_{(15)} = 39.24$, $P < 0.001$, 2011: $\chi^2_{(15)} = 37.48$, $P < 0.01$, and 2012: $\chi^2_{(15)} = 51.0$, $P < 0.0001$).

Vital rates among sites and plots

All three vital rates were markedly size-dependent and both growth and fecundity functions varied markedly among sites, but not among plots within lowland and upland sites (Table 4). Survival probabilities varied neither among sites or plots within-sites (Table 4).

Table 4. The effects of *Size* and *Site* (complete dataset), and *Size* and *Plot* (within sites dataset), including their interactions, on each vital rate function. Growth functions are linear and describe future size (size at $t + 1$), while survival and flowering probabilities are logistic functions. *F* and *Deviance* values are show with degrees of freedom between brackets.

		Vital rate		
		Growth (μ)	Survival Probability (P_{surv})	Flowering Probability (P_{flower})
All	*Size*	$F_{(1)} = 42695^{***}$	$Dev_{(1)} = 60.9^{***}$	$Dev_{(1)} = 595.8^{***}$
	Site	$F_{(1)} = 83.8^{***}$	$Dev_{(1)} = 0.005^{NS}$	$Dev_{(1)} = 24.57^{***}$
	Size × *Site*	$F_{(1)} = 58.7^{***}$	$Dev_{(1)} = 0.173^{NS}$	$Dev_{(1)} = 0.64^{NS}$
Lowland	*Size*	$F_{(1)} = 19646^{***}$	$Dev_{(1)} = 37.9^{***}$	$Dev_{(1)} = 221.9^{***}$
	Plot	$F_{(2)} = 0.15^{NS}$	$Dev_{(2)} = 2.82^{NS}$	$Dev_{(2)} = 1.48^{NS}$
	Size × *Plot*	$F_{(2)} = 0.05^{NS}$	$Dev_{(2)} = 2.56^{NS}$	$Dev_{(2)} = 0.94^{NS}$
Upland	*Size*	$F_{(1)} = 27545^{***}$	$Dev_{(1)} = 22.32^{***}$	$Dev_{(1)} = 384.7^{***}$
	Plot	$F_{(2)} = 1.09^{NS}$	$Dev_{(2)} = 3.72^{NS}$	$Dev_{(2)} = 4.02^{NS}$
	Size × *Plot*	$F_{(2)} = 2.42^{NS}$	$Dev_{(2)} = 1.59^{NS}$	$Dev_{(2)} = 1.92^{NS}$

Significance codes: *** $P < 0.0001$, and ^{NS} $P > 0.05$

Annual mortality and recruitment rates

Mortality rates showed no difference among plots (see Appendix B4; Figure 7A) or sites ($P > 0.05$, $N = 1{,}000$ permutations; Figure 7A). Recruitment rates however, were markedly different, both among sites, and plots within-sites (Figure 7B). The logged plots were the most compromised with the lowland logged plot, L1, presenting fewer recruits than the other lowland plots ($P < 0.001$, $N = 1{,}000$ permutations for both comparisons – Appendix C5), while the upland logged plot, U3, presented no recruits during the studied period. Excluding logged plots, lowland site exhibited the highest recruitment rate ($P < 0.01$, $N = 1{,}000$ permutations), indicating that *B. australis* regeneration was affect not only by logging, but also by altitude.

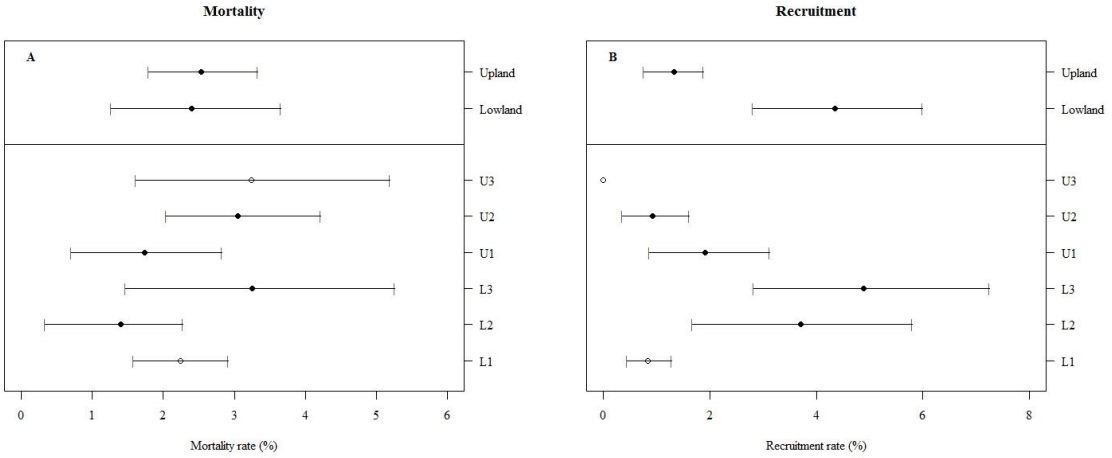

Figure 7. **A)** Annual mortality, and **B)** recruitment rates with bootstrapped 95% confidence intervals (N = 1,000 simulations) for each lowland and upland *B. australis* population plot considering all year's pooled data. Pooled upland and lowland datasets excluded the respective logged plots. Empty circles represent logged plots and filled circles the unlogged ones.

Demographic Models

Even that GLM results indicated for no differences among plots, we selected vital rates regressions for each plot (Table 5) considering the observed variance in recruitment. However, we also selected vital rate regressions for the pooled plots in each site due to the marked effects of site on growth and fecundity. Lowland growth was better described by quadratic functions, while upland growth was better described by linear functions (Table 5, Figure 8 and 9), and lowland models presented higher slopes than upland models, demonstrating faster growth rates for the lowland individuals, but with a slowdown as plants got larger. Flowering probability functions were linear for all plots; however, when evaluated at the site level, flowering probability was better described by quadratic functions. Upland population presented not only earlier (i.e. at a smaller size), but higher flowering, as 80 % of 15 m height individuals were reproductive in the upland against only 60 % in the lowland (Figure 9). Interestingly, during our three years study period, not all large and potentially reproducing individuals flowered, suggesting that although *B. australis* populations have annual reproductive cycles, individuals may display supra-annual cycles, a feature already described for other tropical species (e.g. Pedroni et al. 2002). In spite of the absence of survival differences among plots, the L1 plot survival probability was better described by a quadratic function when all other plots were linear, but this was a consequence of the death of

a 10 m size individual due to termite infestation at L1 when the majority of deaths were concentrated below the three meters size in the lowland and two meters size in the upland.

Although the number of flowering individuals was proportionally higher at the upland, the number of recruits did not correspond to such increased flowering, as demonstrated by the low ratio of recruits per reproductive adult in the upland (Table 6). Besides, the mean recruit size was also higher at the lowland (0.09 ± 0.03 m) than at the upland site (0.07 ± 0.03 m). These together generate a higher fecundity profile at the lowland, which can be seem in the final kernels that were build for each lowland and upland site (Figure 10), and populations plots (Appendix C). In the final kernels we also note the prevalent growth at the lowland, and stasis at the upland (Figure 10).

For both sites population growth rates and confidence intervals were bigger than unit ($\lambda > 1$), suggesting population positive growth at both altitudes (Figure 11). Within sites, the logged plots presented λ equal to unit, suggesting that L1 and U3 populations are in equilibrium while all other populations are growing. The L1 plot even presented lower growth than the other lowland plots (see Appendix B6). Besides the logging effect we also found an altitude effect at the site level analysis since lowland growth rate ($\lambda = 1.084$) was higher than upland growth rate ($\lambda = 1.022$, $P < 0.001$ based on 1,000 permutations), in agreement with the genetic diversity results.

Table 5. Vital rate regressions and parameter estimates used to construct the kernels for the Integral Projection Models (IPM), and resulting population growth rates (λ) with bootstrapped 95% confidence intervals of lowland and upland *B. australis* populations in the Serra do Mar mountain range (SE Brazil) during 2010–2012. The models are functions of plant size (x; height, measured in meters).

Population	Vital rate regressions			Lambda (λ)	95% CI
	Growth (μ)	Survival probability (P_{surv})	Flowering probability (P_{flower})		
L1	$\mu = -0.07 + 1.25x - 0.01x^2$	logit (P_{surv}) = 1.69 + 1.13x - 0.07x^2	logit (P_{flower}) = -8.04 + 0.61x	1.015	[0.987, 1.073]
L2	$\mu = -0.11 + 1.34x - 0.02x^2$	logit (P_{surv}) = 3.26 + 0.15x	logit (P_{flower}) = -7.37 + 0.60x	1.156	[1.100, 1.195]
L3	$\mu = -0.12 + 1.28x - 0.01x^2$	logit (P_{surv}) = 2.25 + 0.28x	logit (P_{flower}) = -6.13 + 0.47x	1.143	[1.087, 1.182]
U1	$\mu = 0.09 + 1.00x$	logit (P_{surv}) = 2.50 + 0.97x	logit (P_{flower}) = -8.26 + 0.83x	1.027	[1.001, 1.042]
U2	$\mu = 0.04 + 1.02x$	logit (P_{surv}) = 2.25 + 0.36x	logit (P_{flower}) = -6.61 + 0.60x	1.021	[1.006, 1.031]
U3	$\mu = 0.09 + 1.02x$	logit (P_{surv}) = 2.11 + 0.30x	logit (P_{flower}) = -7.40 + 0.60x	1.018	[0.978, 1.037]
Lowland	$\mu = -0.08 + 1.27x - 0.01x^2$	logit (P_{surv}) = 2.26 + 0.46x	logit (P_{flower}) = -11.58 + 1.45x – 0.04x^2	1.084	[1.040, 1.101]
Upland	$\mu = 0.07 + 1.01x$	logit (P_{surv}) = 2.33 + 0.39x	logit (P_{flower}) = -9.70 + 1.28x – 0.03x^2	1.022	[1.011, 1.030]

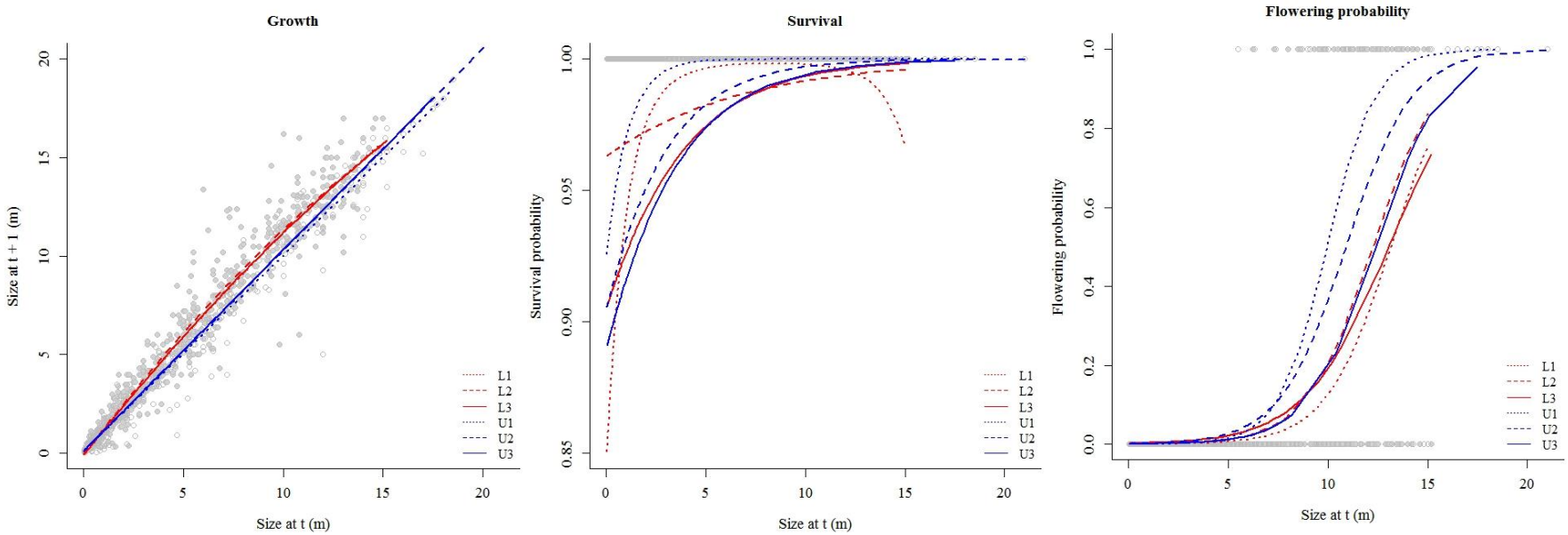

Figure 8. Growth, survival, and fecundity (flowering probability) vital rates regressions with size for each *B. australis* population plot from lowland (red) and upland (blue) sites in the Serra do Mar mountain range, SE Brazil. See Table 5 for regression models description.

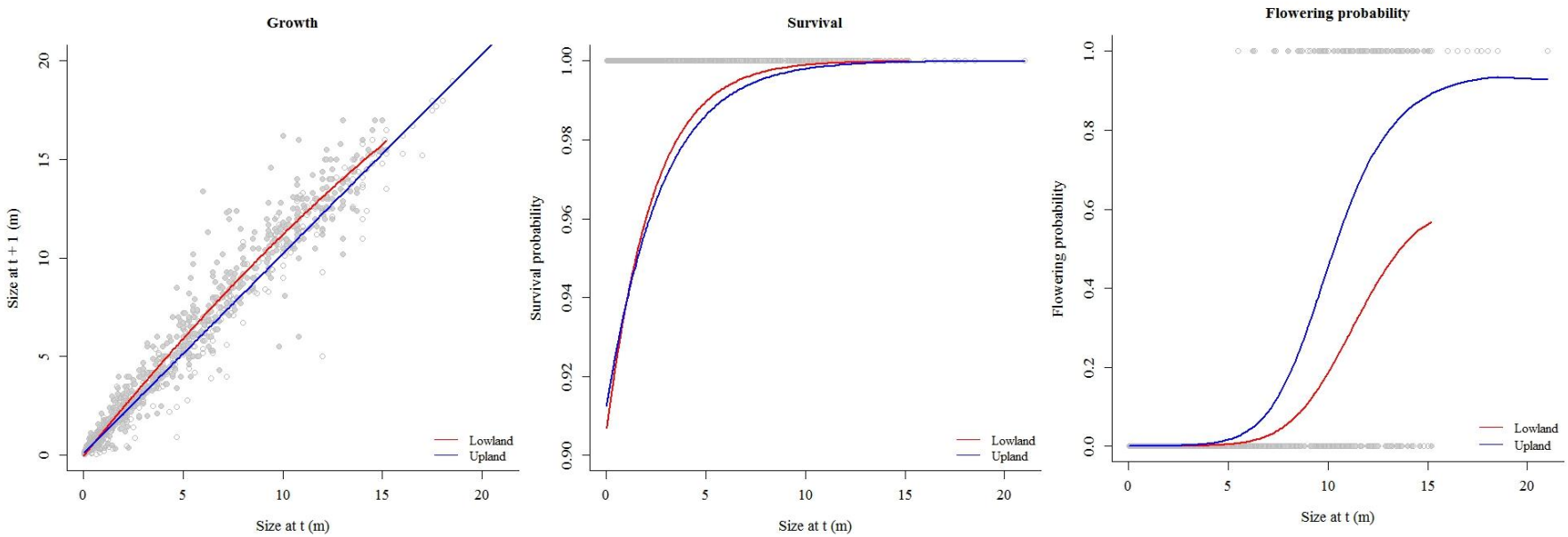

Figure 9. Growth, survival, and fecundity (flowering probability) vital rates regressions with size for pooled *B. australis* populations from lowland (red) and upland (blue) sites in the Serra do Mar mountain range, SE Brazil. See Table 5 for regression models description.

Table 6. Total number of individuals, recruits, and reproductive adults of lowland and upland *B. australis* populations in the Serra do Mar mountain range (SE Brazil) during 2010–2012. The number of established seedlings per reproductive adult (*pe*), and the mean and standard deviation (sd) of recruit size are also shown and were used to parameterize IPM models together with vital rate regressions. Population U3 estimations of *pe*, mean, and sd recruit size were obtained from the whole upland population since U3 population had no recruits during the studied period.

Population	Total number of individuals	Number of recruits	Number of reproductive adults	*pe*	Mean ± sd recruit size (m)
L1	939	15	39	0.385	0.078 ± 0.031
L2	155	11	8	1.375	0.092 ± 0.029
L3	189	17	9	1.889	0.100 ± 0.040
U1	301	11	29	0.379	0.059 ± 0.007
U2	457	8	41	0.195	0.083 ± 0.043
U3	188	0	9	0.240	0.069 ± 0.030
Lowland	1283	43	56	0.770	0.091 ± 0.035
Upland	946	19	79	0.240	0.069 ± 0.030

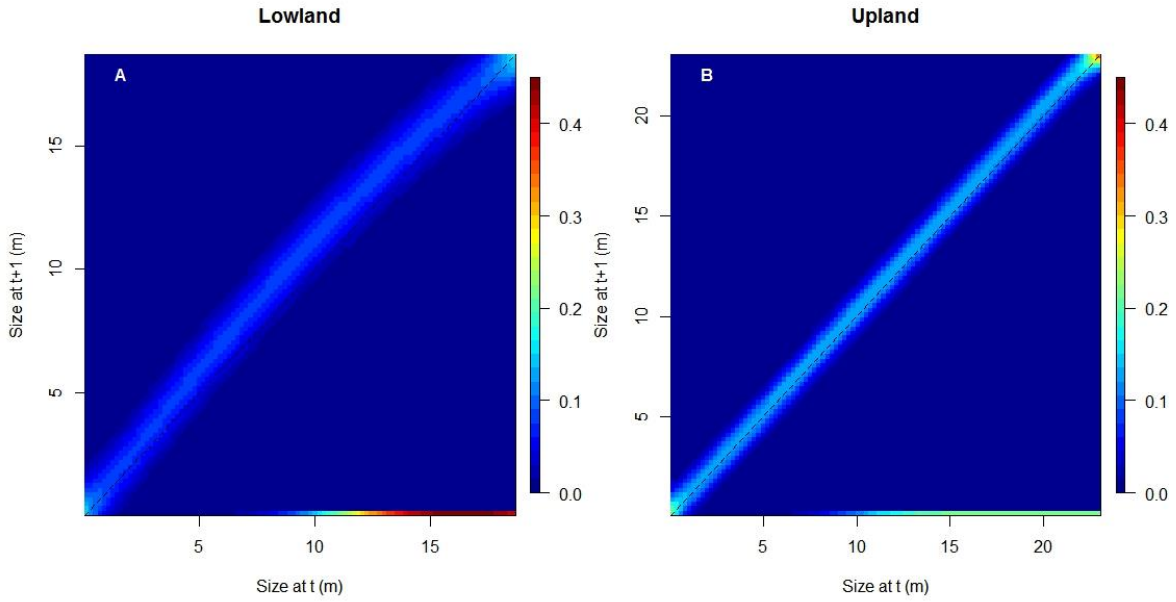

Figure 10. Integral projection models for *B. australis* populations from **A)** lowland, and **B)** upland sites in the Serra do Mar mountain range (SE Brazil). The dashed lines at the principal diagonal are used as reference for the stasis probabilities.

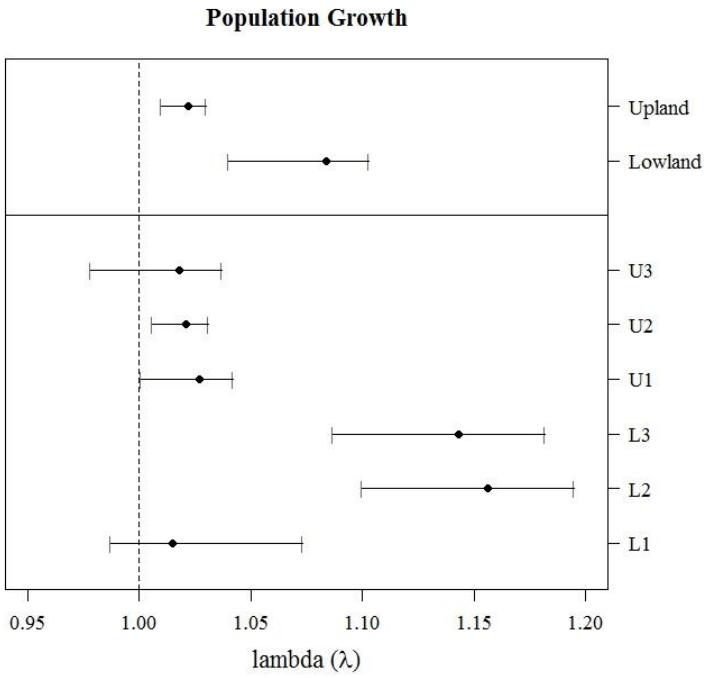

Figure 11. Asymptotic growth rates (λ) with 95% confidence intervals generated from 1,000 bootstrap simulations. The two *B. australis* populations from the logged plots L1 and U3 showed lambda values equal to unit, indicating these populations are in equilibrium. All others populations showed positive asymptotic growth, including the pooled lowland and upland sites. Lowland populations from L2 and L3 plots presented higher λ values than all the others (see Appendix B6).

Elasticity and LTRE analysis

The greatest transition elasticities of λ changes at the lowland site were observed for growth, especially of the smaller size individuals (Figure 12A), while at the upland site we found a highest proportional sensitivity for the stasis transition (i.e. survival without growth), especially of extremely large individuals (Figure 12B).

The LTRE results for the comparison among lowland and upland Δλ pointed out for the higher lowland growth as the main driver for such differences, especially the growth of the smallest individuals (< 30 cm), while upland had the greatest stasis, especially of the smaller and extremely large individuals (Figure 13). Drawing attention to the smallest individuals, we notice

that upland growth profile consisted of small size increments and stasis, while lowland exihibited much greater size increments. The higher lowland fecundity also played a role (Figure 13).

For the λ differences observed among lowland logged and non-logged plots, the LTRE pointed out for the greatest stasis (and lower growth) of small size individuals and lower fecundity in the logged plot as the main responsible (Figure 14). As the flowering probabilities were similar among logged and non-logged lowland plots, the fecundity differences encountered between treatments were caused by the lower recruitment at the logged plot.

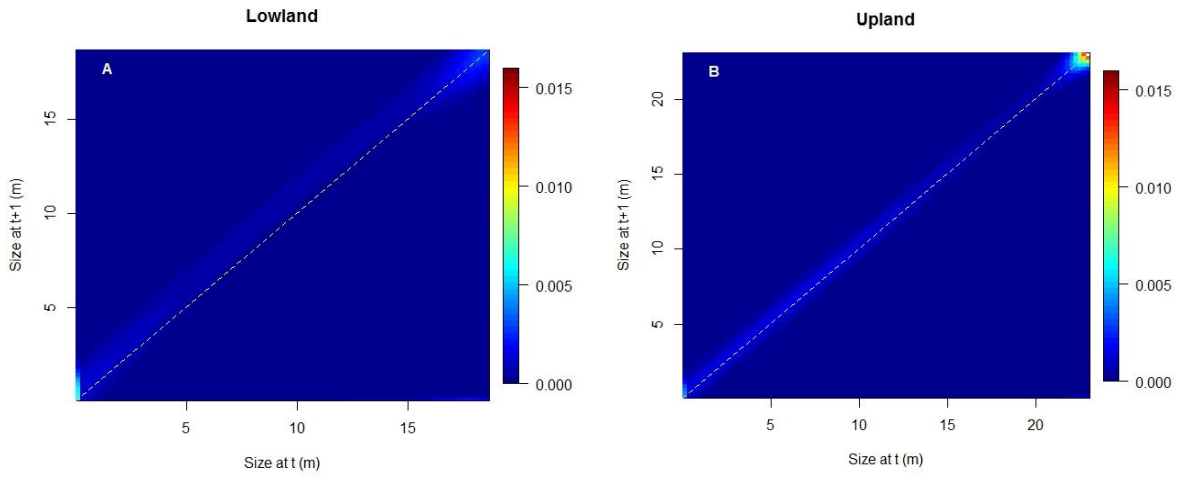

Figure 12. Matrix elasticity of population growth rate (λ) for combined **A)** lowland, and **B)** upland *B. australis* populations. The dashed lines at the principal diagonal are used as reference for the stasis probabilities.

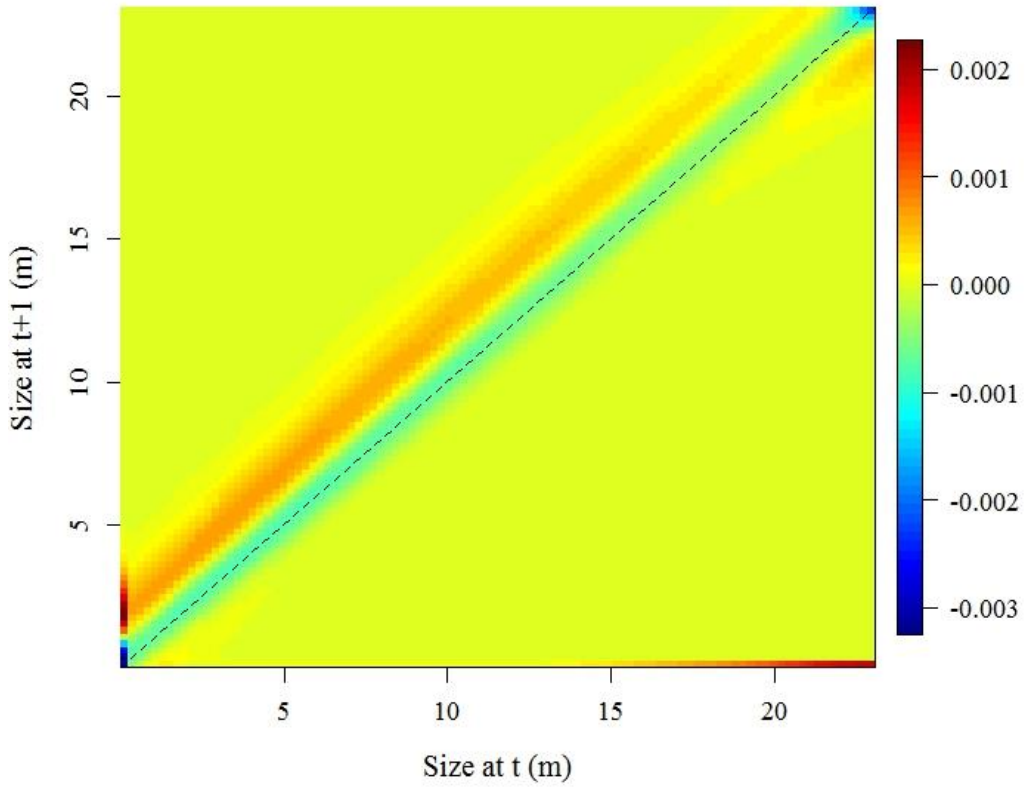

Figure 13. Life table response experiments results. The contribution of matrix transitions to Δλ in the comparisons among *B. australis* populations from lowland (treatment) × upland (control). The dashed lines at the principal diagonal are used as reference for the stasis probabilities.

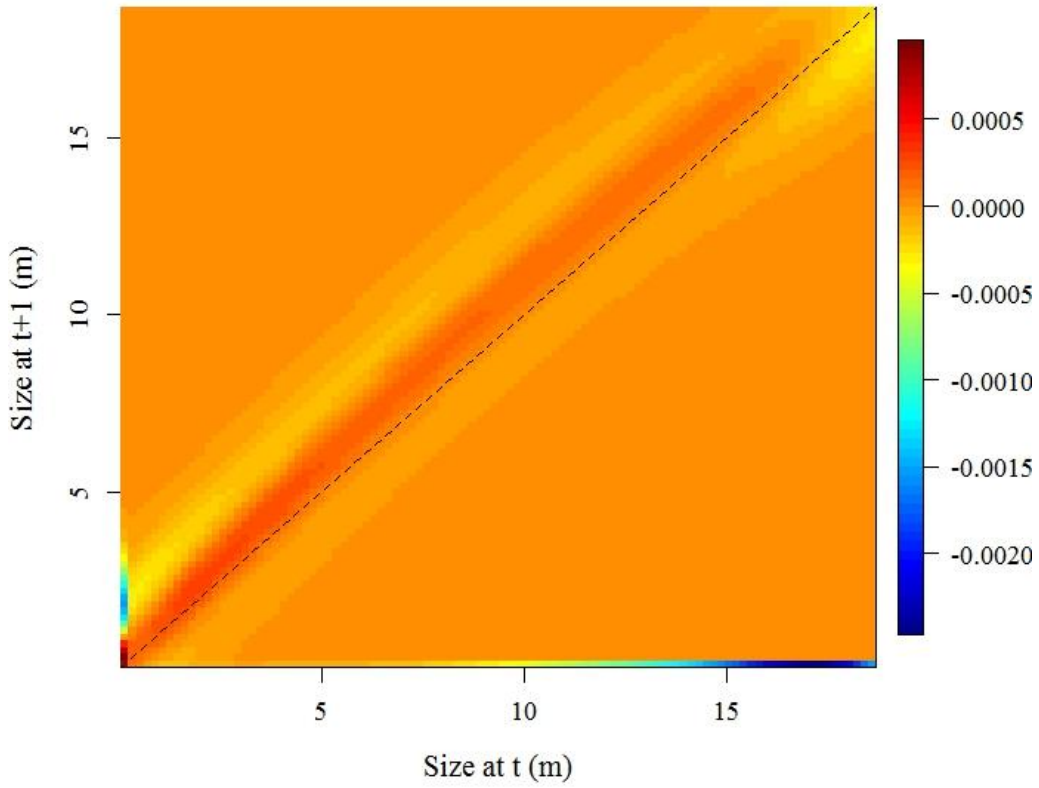

Figure 14. Life table response experiments results. The contribution of matrix transitions to Δλ in the comparisons among *B. australis* populations from logged lowland (treatment) × non-logged lowland (control). The dashed lines at the principal diagonal are used as reference for the stasis probabilities.

Discussion

Logging on Bathysa australis structure and demography

The continuous extraction of selected trees from forest stands as a mean of economic exploitation of wood products usually has major structural consequences for the remaining forest by intensifying the natural dynamics of gap formation (Pereira et al. 2002, Asner et al. 2004). The effects consist of significant small-scale disturbances for the forest stand, including the effects on canopy, on the lower strata and also on the forest floor if roots are uplifted (Uhl & Vieira 1989). The gap formation has its consequences, such as increased light availability, air and soil temperatures, and wind exposure (Denslow et al. 1998).

Considering this, and acknowledging that the logged plots of our study site were the ones with more gaps.area^{-1} (see Eisenlohr et al. 2013), they might have increased light availability with improved opportunities for flowering, seed germination, recruitment and diameter growth, resulting in higher fecundity, density and biomass. This would be true especially because *B. australis* presents some important biological features, such as dependence on light for germination, as reported by Duz et al. (2004). Following the initial growth of *B. australis* seedlings (height < 5 cm) at different light environments, they not only observed that its seeds do not germinate in the dark, but also noted a biomass increase at higher light availability, which was a consequence of diameter rather than height growth.

In agreement with the assumption of increased light availability, the lowland logged plot presented higher density and total basal area than the unlogged plots, but fecundity was lower though. If we think the lowland site as a high-competitive environment, were resource-factors such as light and nutrients are limiting species growth, we can understand such increased density and basal area as a consequence of the competition release that happens after the opening of gaps. *B. australis* is a plant that can take advantage of such opportunities for growth and recruitment (see Duz et al. 2004), and the structural changes that came with the past selective logging must have favored the spread of this species.

However, fecundity was lower at the logged lowland plot and this was pointed as one of the causes for the lower population growth. Since the probability of flowering was the same for both logged and non-logged plots, we believe such fecundity differences are due to lower seed set or due to local environmental restrictions that could have affected germination or establishment. Decreases in seed set could have emerged through the low of effective pollination, but this has a low probability of occurring since the other lowland plots are too close (less than 1 km) to be affected by pollinators in different ways. Local environmental restrictions, on the other hand, are most likely to have caused such recruit depletion since logging has probably done major modifications on stand structure, dynamics and abiotic conditions. Besides, although most plant populations are seed limited, it has been shown that establishment limitation, rather than seed limitation, is the most important factor limiting the distribution and abundance of individuals (Clark et al. 2007). Among the various kinds of environmental restrictions, we can cite the current high population density as a potential inhibitor for the entering of new recruits. We should note that the *B. australis* seeds are autochoric, and the majority of the seed rain falls near

the parent plant due to the absence of a dispersal vector, which has been demonstrated by the strong spatial genetic structure (Reis et al. 2015). Density-dependent seed predation, herbivory, or seedling mortality are then all very plausible explanations, although density-independent mortality due to desiccation, for example, cannot be discarded. Considering that the smaller individual's growth and stasis were also recognized as driving the observed λ differences among lowland logged and non-logged plots, we expect that mortality sources at the early stages in the life cycle are of great importance for the successful performance of *B. australis* populations.

At the upland, recruitment was also reduced at the logged plot, but density and basal area were compromised as well. Perhaps, at the upland light availability is not as limiting for growth and recruitment as in the lowland due to the greater number of canopy openings caused by small-scale disturbances and the higher slopes. The latter usually increases forest canopy heterogeneity allowing for the incidence of lateral and scattered light. In addition, we must acknowledge the fact that the logged plot had the lowest slope (measured as altitudinal variation) at the upland (U1: 6.4 ± 2.6 m, U2: 5.6 ± 1.5 m, U3: 4.0 ± 1.1 m; unpublished data), and this might be confounding the effects. When slopes are steep, there is less litter accumulation on the forest floor due to rain carry over. This would release *B. australis* seeds from the effects of deep litter layers, such as the shading that inhibits germination of light demanding seeds, and the physical barrier for the emergence of seedlings (Vázquez-Yanes et al. 1990, Molofsky and Augspurger 1992). Thus, the logged plot must have the deepest litter layer at the upland, inhibiting seeds germination and seedling emergence, and explaining the lack of recruits during our study period.

Concerning the multi-stemmed frequency, we found a pattern of increased multi-stems at the logged plots, both at lowland and upland sites. This result is consistent with the whole community pattern and suggests the occurrence resprouting responses to the damage caused by disturbances. As noted by Eisenlohr et al. (2013), among all studied plots along the elevation gradient, the logged ones had more multi-stemmed plants in the community.

Survival and height growth, on the other hand, were not affected by logging neither at the lowland or the upland, but this is probably an outcome of the increased resprouting capacity that *B. australis* presents, reallocating resources to recover aerial parts that were lost by means of damage. Besides that, *B. australis*, during its initial development, presents great plasticity in the use of the light environment in a way that, even at shading conditions seedlings are capable of improving light absorption by increasing the leaf area ratio and decreasing root/shoot ratio,

keeping positive relative growth rates and perpetuating in the environment (Duz et al. 2004). At the upland, size structure was not affected by logging either, but at the lowland we observed a lower frequency of small size individuals at the logged plot compared to the unlogged ones. As mentioned above, this is probably an outcome of the lower recruitment rates caused by local environmental restrictions.

In conclusion, even that both *B. australis* populations at the logged plots had suffered significant changes in structure and dynamics, only the lowland population presented truly significant changes in performance among logged and non-logged plots. In the upland, although the population growth rate of the logged plot was equal to unit, denoting equilibrium, while the other two plots were growing ($\lambda > 1$), these three upland plots were not significantly different from each other. The lowland logged plot, on the contrary, presented a significant λ reduction compared to the unlogged ones. The positive effects on density and basal area did not compensate for the negative effects on fecundity and on smaller individual's growth, the main drivers of the observed performance differences. Actually, we believe that it might have been some positive effects of logging on recruitment in the past, generating the observed high density and biomass, but then such increased intraspecific biomass might be inhibiting current recruitment and small size individuals growth. Although we do not have detailed information regarding the procedures for timber extraction, and the impact intensity, we do know from local people when approximately such activities ceased. Perhaps, the ten more years of timber extraction at the lowland might explain such differences in logging impacts among altitudes, since the upland had more time for forest recovery.

Elevation on Bathysa australis structure

In the upland, the lower temperature conditions together with a high disturbance frequency would restrict growth, generating smaller size structures, and promoting the resprouting strategy for the surviving damaged individuals. Indeed, the frequency of multi-stemmed individuals matched these expectations with greater values for the upland. This site usually presents the highest slopes where terrain is more unstable (Poorter et al. 1994), and exposed to wind blow, therefore subject to frequent small-scale disturbances. In addition, the lower productivity of upland sites (Bruijnzeel & Veneklaas 1998, Girardin et al. 2010) usually means restricted individual growth (e.g. Coomes and Allen 2007), and in high productive sites,

such as the lowland, where height growth is unrestricted, the multi-stemmed architecture is usually not advantageous since it promotes more shading (Bellingham and Sparrow 2009). Except when subject to frequent disturbances multi-stemmed architecture might be advantageous at this high productive sites, as we could see for the lowland logged plot.

However, the height distributions were apparently similar among altitudes. Even though lowland plants presented faster growth rates than upland ones, after reaching ~10 m high they also displayed a marked slowdown on growth and this is probably the explanation of why they did not achieved higher sizes than upland plants. On the contrary, upland population presented a few individuals larger than the maximum height observed at the lowland (17 m against 21 m at the upland) due to the lack of such forces that limited height growth on lowland plants after they got larger. Such forces remain unexplained.

In the same way, the total basal area, our surrogate for biomass, was not affected by altitude. Similar height distribution with similar total basal area leads us to believe that *B. australis* biomass was not really affected by altitude at this elevation gradient. In fact, while tropical elevation gradients usually display significant declines in aboveground biomass, the elevation gradient of the Serra do Mar mountain range goes in the opposite direction towards increased biomass stocks with elevation (Alves at al. 2010). Although such biomass increase was not the case of *B. australis* populations, still is a notable exception to the overall pattern. Density estimates, on the other hand, were affected by altitude with greater individual counts observed for the upland, which is in accordance with the majority of reports of *B. australis* abundance at high- and low-elevation sites (e.g. Arzolla 2002, Leite and Rodrigues 2008, Padgurschi et al. 2011, Pereira 2011, Sanchez et al. 2013, Stefani 2013), and also with the negative correlation between where the species is most common and where it grows the best (McGill 2012).

Similar basal area sums with lower lowland density would imply in three possible explanations that could compensate for the greater upland density and balance total basal area: 1) more multi-stemmed individuals at the lowland site, 2) more recruits (small size individuals) at the upland, and 3) size structure with relatively larger individuals at the lowland. As already mentioned above, none of these possibilities matches the observed findings. The lowland site presented higher recruitment rates, with less multi-stemmed individuals than the upland, and size structure was similar among altitudes. However, we should note that only height distributions are

presented, and although the height scale is strongly correlated with diametric scale, may always be some differences.

Especially at elevation gradients, differences concerning height-diameter allometric relations are always expected. Precisely, we would imagine lowland plants to have higher height growth in proportion to diameter than upland ones since lowland plants are usually in a more shaded and wind protected environment. At our study site, lowland plants are probably more shaded too because of the higher canopy (see Scaranello et al. 2012), and because upland slope allows for the incidence of lateral light. However, *B. australis* allometry has not shown such greater height investment in lowland plants, but the opposite pattern (unpublished data). Upland plants that were bigger than five meters height presented higher line-fitting slopes indicating for greater height investment while lowland plants appeared to have relatively more diameter growth. Although such findings contradict the overall expectations for elevation gradients, it can explain the balanced biomass among altitudes. Greater diameter growth in lowland plants compensates for the higher upland densities. Besides, such higher density at the upland probably increases shading by neighboring plants and provides wind protection in a way that could also explain the height growth investment at this site.

Elevation on Bathysa australis demography

In this study we evaluated the demographic fitness of a wide-range tree species at two extreme sites in the elevation gradient of the Serra do Mar mountain range, SE Brazil. We found a higher population growth rate for the lowland population of *B. australis*, in agreement with the previously reported higher genetic diversity, thus supporting the theoretical prediction of positive correlation among genetic diversity and fitness.

There are two possible ways, in two different directions that fitness and genetic diversity could be positively correlated, both involving the effects of population size at some point (Reed & Frankham 2003, Leimu et al. 2006). First, when reductions in population size decrease genetic variation (e.g. via genetic drift), and these implicate in less mates to reproduce and higher inbreeding, these will probably reduce fitness as well. Second, when habitat quality is different among populations and this variation is reflected on fitness (e.g. via selection) in a way that population sizes are also affected, then the amount of genetic diversity will probably be influenced either. Our earlier findings did not show any evidence for the occurrence of

bottlenecks at the upland *B. australis* population that could suggest a past reduction in population size as the start of subsequent cascade effects on diversity and fitness (see Reis et al. 2015). However, the two sites differ markedly in habitat quality and this could have been the initial fuel for the subsequent effects on fitness and genetics. Thus, this conformity among *B. australis* genetic diversity and fitness might reveal an effect of demography on genetics rather than the contrary, but this can only be assured by comparing the field results with a common garden experiment that eliminates the effect of habitat quality (Leimu et al. 2006).

In spite of *B. australis* greater fitness at the lowland, density was lower at this site. Tthis pattern of higher estimated abundances (> 10 indiv.ha^{-1}) on the upper slopes (e.g. Arzolla 2002, Leite and Rodrigues 2008, Padgurschi et al. 2011, Pereira 2011, Sanchez et al. 2013, Stefani 2013), and lower densities on the lower ones (e.g. Moreno et al. 2003, Assis et al. 2011, Campos et al. 2011, Gomes et al. 2011, Prata et al. 2011, Sanchez et al. 2013) is in fact more frequently found for this species, although there are exceptions (e.g. Gomes et al. 2011, Ramos et al. 2011, Stefani 2013). However, we could say that this contradictory pattern among abundance and fitness is not as contradictory as it seems. There is interesting evidence for the eastern North America trees that plants are rarely most abundant were they perform best, and this is probably an outcome of the trade-off among a species competitive ability and environmental tolerance (McGill 2012). The author suggests that the exclusion of a less competitive, but tolerant species from its optimal environment by a dominant better competitive species might explain the observed negative correlation between where the species is most common and where it grows the best. We can certainly think of *B. australis* as a tolerant species, as suggested by its wide range; and the notably heterogeneity found on tropical forests such as our study site might perfectly allow for the maintenance of populations of such less competitive species. Considering that, a possible explanation is that *B. australis* abundance is somewhat suppressed by other better competitive species in the lowland, where it performs the best, while in the upland this species displays a relatively higher mean performance when compared to the other species in the community, behaving as a better competidor and thus maintaining the observed higher density estimates.

The fitness reduction observed for the *B. australis* upland population is not an exception, as there are further studies reporting lower performances for other species populations at high-elevation sites (e.g. Kim & Donohue 2011). Individual growth (Grant & Mitton 1979, Fernández-

Calvo and Obeso 2004, Coomes and Allen 2007, King et al. 2013), fruit and seed set (Eriksen et al. 1993, Hemborg and Karlsson 1998, Brito and Sazima 2012), and seedling numbers (Cierjacks et al. 2008) are some of the performance components that have been described to suffer significant reductions at high-elevation sites. For *B. australis*, both growth and fecundity patterns have also been compromised at the upland population in the Serra do Mar mountain range. Especially for the smallest individuals, growth was disparate among sites and pointed as the main driver of lowland and upland demographic differences.

The size of individuals during their inicial phases of development (e.g. seedlings and saplings) is directly linked to their survival, as larger sizes allows for higher photosynthetic areas, higher tissue toughness preventing herbivory, and reduced chances of dying from desiccation (Kitajima & Fenner 2000). This might probably be the case of *B. australis*, whose seeds are too small to have any resources to rely on. In fact, survival was size-dependent on the overall *B. australis* populations, with 80 % of deaths concentrated until 1 m high individuals. In this sense, marked size increments at this early stage might represent a significant advantage for survival probabilities over other plants in the community, and this must be especially true for the highly competitive lowland site. This can be illustrating how different selective pressures from two different environmental contexts could drive the same species to perform distinct growth strategies. Upland population presented much lower size increments compared to lowland at early stages, but these remained fairly constant along the onthogeny, while lowland population had the greatest growth at the beginning but slowed down as plants got larger. However, both sites had similar survival patterns.

Fecundity also seemed to contribute for the observed differences on fitness among altitudes. Fecundity differences are probably associated with the lower flowering probabilities and higher establishment rate (*pe*) observed at the lowland. Lowland presented not only the highest abundance of recruits, but also the lowest abundance of reproductive adults. This result suggests that the upland population has done major efforts in reproduction, through its earlier flowering and increased number of flowering individuals, which was not accompanied by equivalent recruitment rates. Our findings are in agreement with literature reports also describing increased reproductive efforts at high-altitude populations that were not followed by equivalent regeneration output (i.e. fruit and seed set, seedling number) when compared to low-altitude populations (e.g. Eriksen et al.1993, Hemborg and Karlsson 1998, Fabbro and Körner 2004). In

our study site, the Serra do Mar mountain range, similar findings were observed for the shrub species *Tibouchina pulchra* Cogn (Melastomataceae), with denser flowering at the high-altitude populations, but restricted fruit and seed set due to low pollinator abundance (Brito and Sazima 2012). *B. australis* flowers are mostly pollinated by social bees and wasps (Freitas and Andrich 2013), small insects that usually fly over short distances (Dick et al. 2008) and whose abundance is probably affected by altitudinal variation, thus compromising *B. australis* reproductive success at the upland. Besides, the already mentioned higher upland density migh also reduce recruitment at this site through density-dependent processes.

Individual growth at the lowland and stasis at the upland also presented a marked contribution to fitness variation among elevation sites. The increased rates of individual growth in the low-altitude population agreed with the majority of literature reports (e.g. Fernández-Calvo and Obeso 2004, Coomes and Allen 2007, King et al. 2013). The decline in growth rates with altitude is usually related to reduced air and soil temperatures and limited nutrient supply (Grubb 1977). Indeed, at the elevation gradient of the Serra do Mar mountain range, an altitude increase has been associated to prominent changes in temperature (Souza Neto et al. 2011, Eisenlohr et al. 2012); however, there is no evidence of such reduction in nutrient availability (Martins 2010). Although soil type and nutrient supply do not change along the gradient, the slope terrain is significantly higher at the upland site that is also more wind exposed. Those factors can favor the occurrence of frequent small-scale disturbances limiting height growth and promoting a more conservative strategy that privileges survival instead of growth. Although we have evidences that mortality is usually higher at the upland tree community - the total basal area of standing dead trees was higher at this site than at any other site in the elevation gradient (Eisenlohr et al. 2012) - *B. australis* survival has not changed among altitudes. Stasis was greatest at the upland site though. These findings reinforce the theoretical predictions of high-elevation sites as stressful environments, where factors such as temperature, wind, and disturbances usually limit growth (Grime 1977, Callaway et al. 2002). According to Grime's theory (Grime 1977), plants subject to harsh environments present slow growth rates and traits that favor survival, while plants growing in productive environments are inherently fast growing. However, even though *B. australis* has shown lower growth at the upland when compared to the lowland, this species strategy of greater flowering and survival seems to be enough for the successful establishment at this high-elevation site.

In the Serra do Mar mountain range, *B. australis* presented a significant demographic plasticity, as it seemed to display different strategies at different altitudes, both resulting in positive population growth rates. This probably occurred because lowland and upland represent different environmental contexts with different selective pressures. The relative demographic stability, with lower but constant growth observed at the upland seems to be enough for the successful establishment of *B. australis* at this less-competitive site. On the other side, the fast dynamics observed at the lowland, with higher recruitment rates and a marked growth at the early stages, but with a slowdown at the latter ones might represent a significant advantage for *B. australis* plants at the highly competitive lowland site. Such differences in dynamic behavior might also in part explain the observed differences in genetic diversity. Since upland population turnover is low, compared to the lowland, the entering of new genotypes is presumably low too. However, in a demographic perspective both strategies can be considered successful as they maintain populations above the substitution rate.

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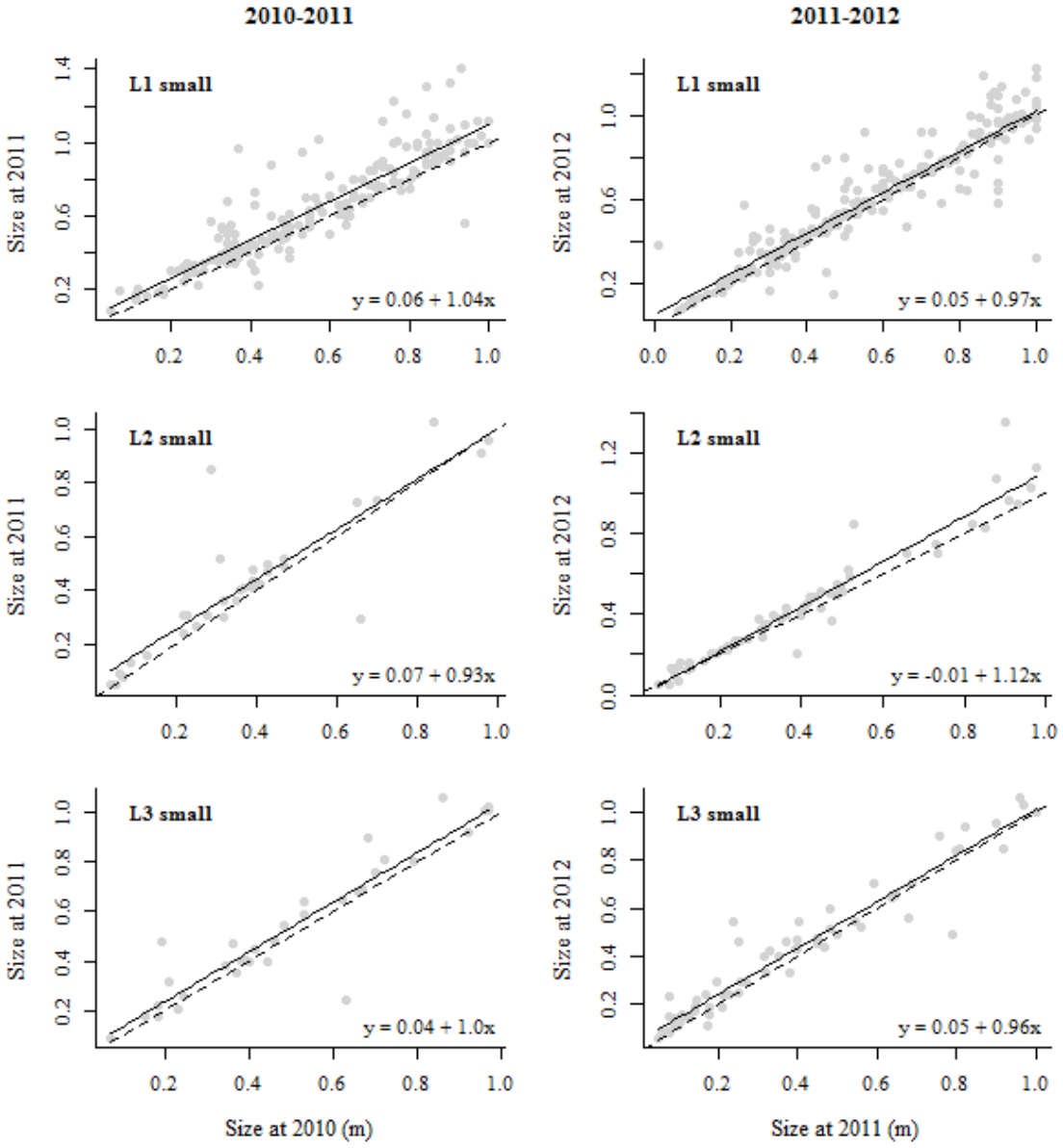
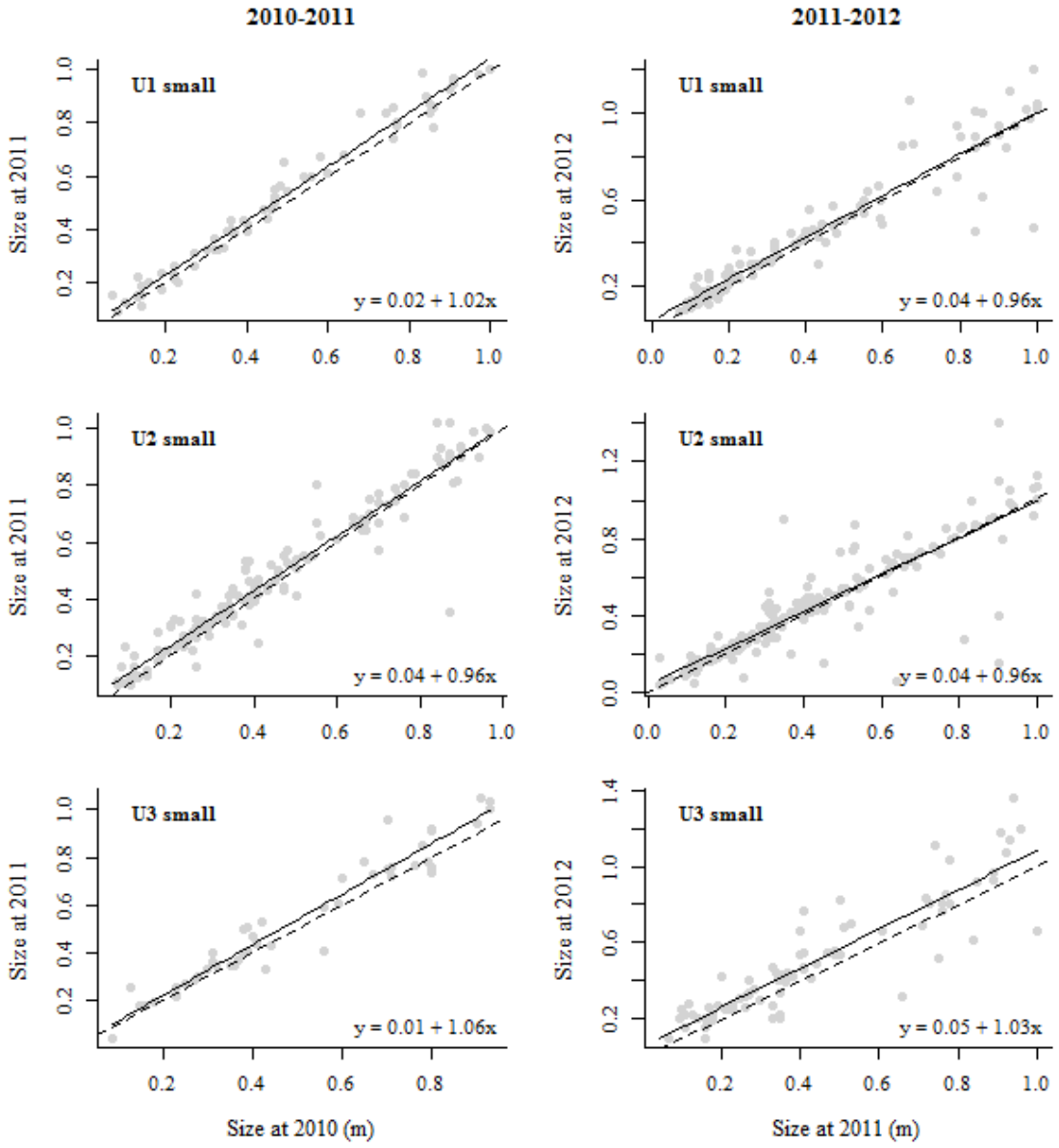
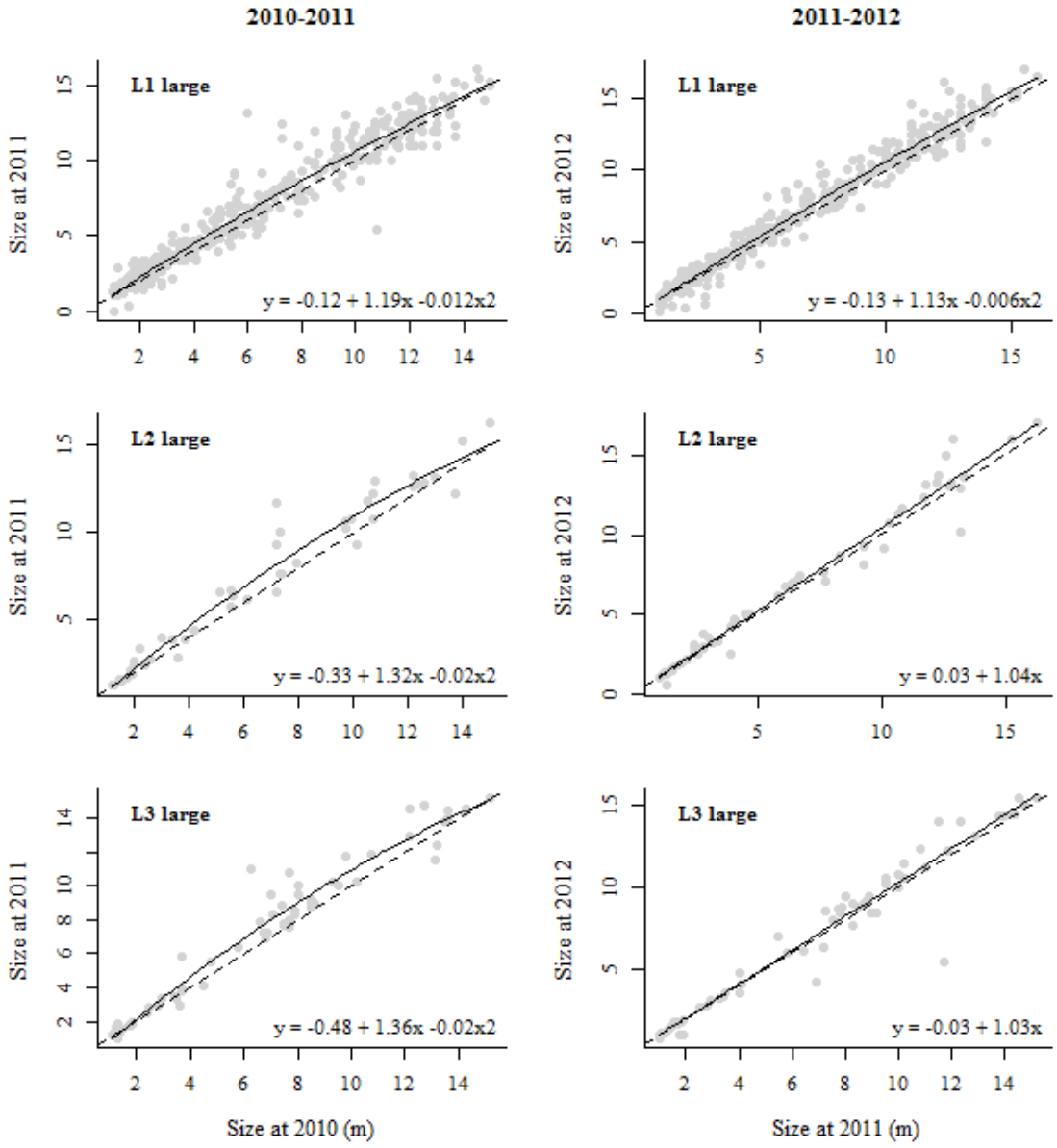
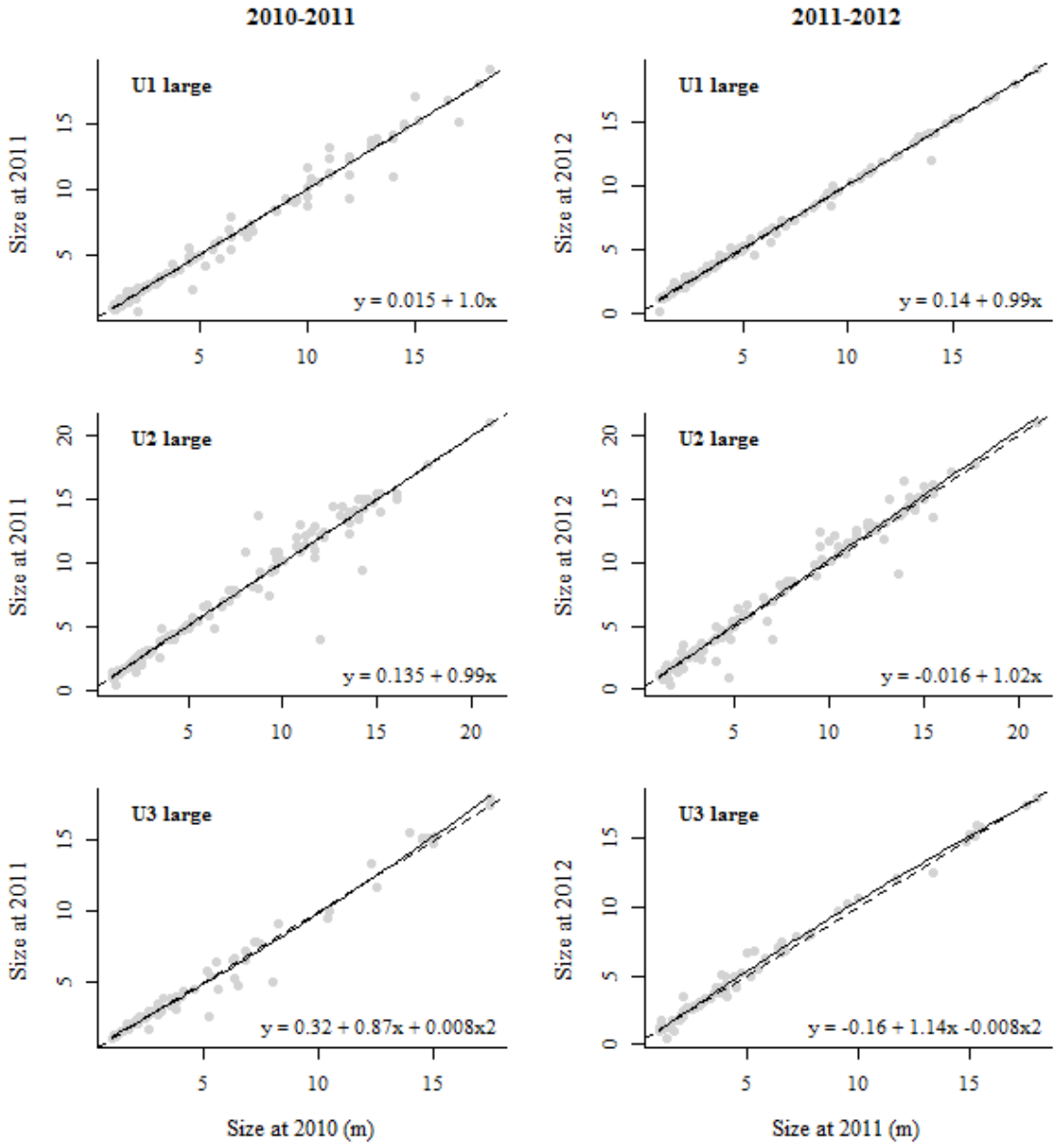
Appendix A

Figure A1. Linear functions describing mean growth according to size for each plot, transition year, and size class (small: ≤ 1 m height, and large: > 1 m height). These functions were used to estimate previous census measures of *false recruit* plants, and therefore distinguish among recruits and pseudo-recruits.




2010-2011
2011-2012
U1 small
y = 0.02 + 1.02x
U1 small
y = 0.04 + 0.96x
U2 small
y = 0.04 + 0.96x
U2 small
y = 0.04 + 0.96x
U3 small
y = 0.01 + 1.06x
U3 small
y = 0.05 + 1.03x
Size at 2011
Size at 2012
Size at 2010 (m)
Size at 2011 (m)


2010-2011
2011-2012
L1 large
y = -0.12 + 1.19x -0.012x2
Size at 2011
L1 large
y = -0.13 + 1.13x -0.006x2
Size at 2012
L2 large
y = -0.33 + 1.32x -0.02x2
Size at 2011
L2 large
y = 0.03 + 1.04x
Size at 2012
L3 large
y = -0.48 + 1.36x -0.02x2
Size at 2011
Size at 2010 (m)
L3 large
y = -0.03 + 1.03x
Size at 2012
Size at 2011 (m)


2010-2011
2011-2012
U1 large
y = 0.015 + 1.0x
U1 large
y = 0.14 + 0.99x
U2 large
y = 0.135 + 0.99x
U2 large
y = -0.016 + 1.02x
U3 large
y = 0.32 + 0.87x + 0.008x2
U3 large
y = -0.16 + 1.14x -0.008x2
Size at 2011
Size at 2012
Size at 2010 (m)
Size at 2011 (m)

Appendix B

Table B1. Density comparisons among plots using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between plots. Only 2012 results are shown because there was no difference between years.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	747^{***}	720^{***}	670^{***}	468^{***}	722^{***}
L2		-	27^{NS}	140^{NS}	279^{**}	25^{NS}
L3			-	113^{NS}	252^{**}	2^{NS}
U1				-	139^{NS}	115^{NS}
U2					-	254^{**}
U3						-

Significance codes: ^{NS} Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table B2. Total basal area ($m^2.ha^{-1}$) comparisons among plots using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between plots. Only 2012 results are shown because there was no difference between years.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	64.98^{**}	60.92^{**}	61.34^{***}	56.31^{***}	66.64^{**}
L2		-	4.06^{NS}	3.64^{NS}	8.67^{NS}	1.66^{NS}
L3			-	0.42^{NS}	4.61^{NS}	5.72^{NS}
U1				-	5.03^{NS}	5.3^{NS}
U2					-	10.33^{NS}
U3						-

Significance codes: ^{NS} Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table B3. Percentage of multi-stemmed individuals (%) comparisons among plots for pooled year data using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between plots.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	8.6^{***}	10.1^{***}	4.7^{**}	6.7^{***}	7.7^{***}
L2		-	1.5^{NS}	3.9^{NS}	1.9^{NS}	16.3^{***}
L3			-	5.4^{*}	3.4^{NS}	17.8^{***}
U1				-	2.0^{NS}	12.4^{***}
U2					-	14.4^{***}
U3						-

Significance codes: ^{NS} Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table B4. Annual mortality rate (%) comparisons among plots for pooled year data using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between plots.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	0.84^{NS}	1.01^{NS}	1.07^{NS}	0.5^{NS}	1.0^{NS}
L2		-	1.85^{NS}	0.34^{NS}	1.65^{NS}	1.84^{NS}
L3			-	1.51^{NS}	0.2^{NS}	0.01^{NS}
U1				-	1.31^{NS}	1.5^{NS}
U2					-	0.19^{NS}
U3						-

Significance codes: ^{NS} Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table B5. Annual recruitment rate (%) comparisons among plots for pooled year data using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between plots.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	2.87 ***	2.87 ***	1.07 NS	0.09 NS	0.84 NS
L2		-	1.18 NS	1.8*	2.78***	3.71***
L3			-	2.98**	3.96***	4.89 ***
U1				-	0.98 NS	1.91*
U2					-	0.93 NS
U3						-

Significance codes: NS Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

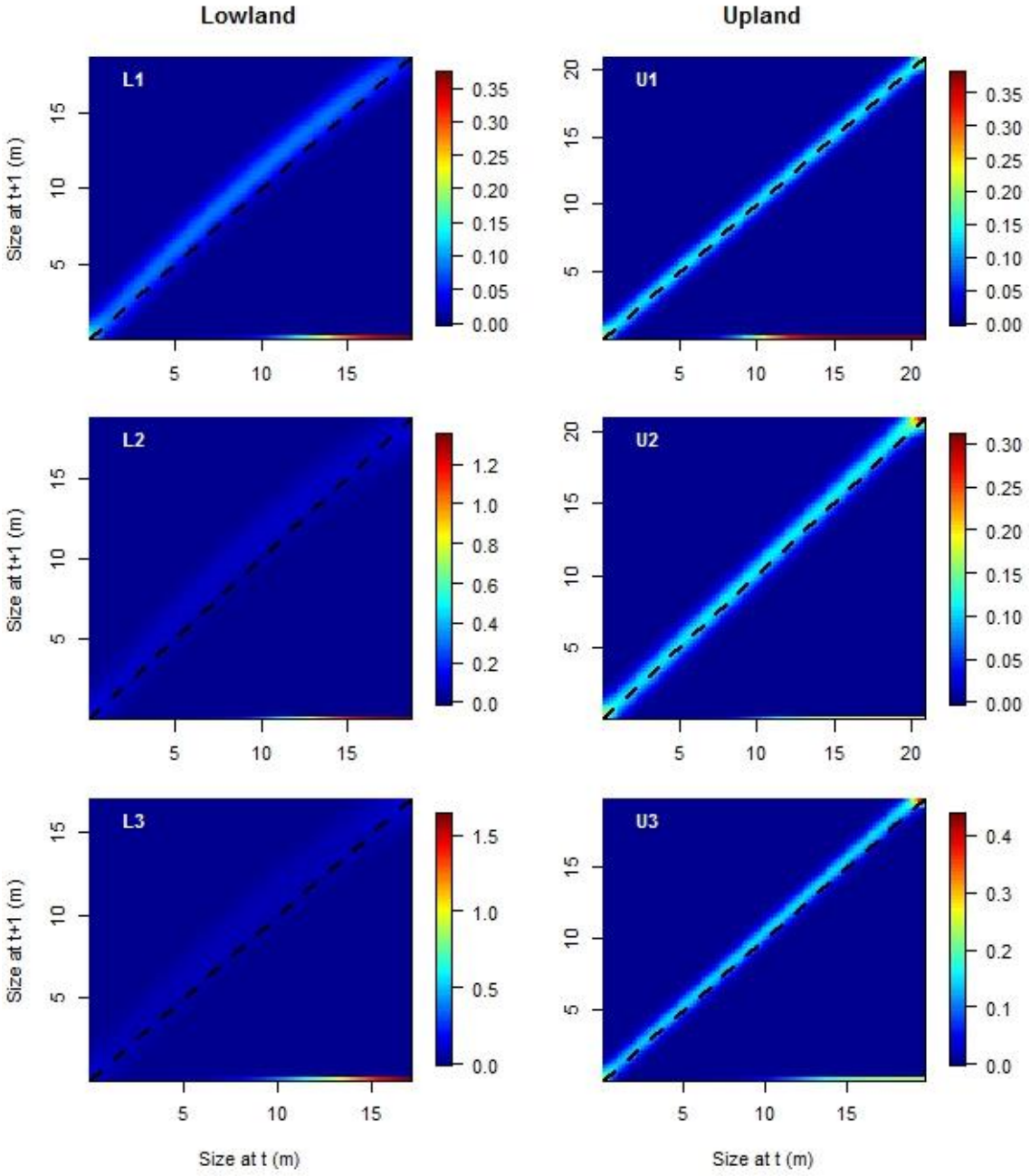
Table B6. Population growth rate (λ) comparisons among plots using a label permutation procedure (N = 1,000). The values correspond to the absolute observed differences between lambdas.

	L1	**L2**	**L3**	**U1**	**U2**	**U3**
L1	-	0.141***	0.128**	0.012 NS	0.006 NS	0.003 NS
L2		-	0.013NS	0.129***	0.135***	0.138***
L3			-	0.116*	0.122*	0.125*
U1				-	0.006 NS	0.009 NS
U2					-	0.003 NS
U3						-

Significance codes: NS Non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Appendix C

Figure C1. Integral projection models for *B. australis* lowland, and upland population plots in the Serra do Mar mountain range (SE Brazil). Note the different scales in the six figures.



CONSIDERAÇÕES FINAIS

Neste trabalho nos propusemos a avaliar populações de uma espécie arbórea (*Bathysa australis*) de ampla distribuição localizadas em posições extremas do gradiente altitudinal da Serra do Mar (SE Brasil) utilizando uma abordagem conjunta de genética e ecologia populacional. Integrando essas disciplinas em uma escala regional, fizemos a tentativa de incluir o processo de migração, que atua além da escala local e é normalmente negligenciado nos estudos populacionais. No entanto, apenas com estimativas indiretas do fluxo gênico histórico já pudemos perceber que mesmo num contexto de floresta contínua, sem nenhuma barreira física aparente, as populações de *B. australis* localizadas nos extremos do gradiente altitudinal estudado estavam isoladas a uma distância inferior a 7 km, já que o fluxo gênico entre estas populações foi extremamente baixo. Embora não tenhamos encontrado um padrão de isolamento-pela-distância explicando a ausência de troca gênica observada, a diferença altitudinal foi apontada como provável responsável. Dois agrupamentos genéticos foram então reconhecidos: um para a fitofisionomia de FOD Submontana (80-216 m) e outro para a de FOD Montana (1010-1100 m), sugerindo que, ao invés das iniciais seis populações amostradas, nós tínhamos apenas duas unidades populacionais do ponto de vista genético. Desta maneira, foi possível responder a segunda e terceira perguntas deste trabalho ao concluir que a marcada estrutura genética observada - configurando ausência de dispersão entre as populações de *B. australis* dos extremos altitudinais - sugere que a escala local seja suficiente para entender os processos dinâmicos responsáveis pela abundância desta espécie.

Estes resultados demonstram a força que os gradientes de altitude podem exercer sobre as populações de plantas, pois além de constatar esta forte estruturação genética, verificamos ainda diferenças nos padrões ecológicos entre as populações das diferentes altitudes. *B. australis* apresentou considerável plasticidade demográfica no gradiente estudado, pois diferentes estratégias foram reconhecidas nas populações Montana e Submontana, sendo ambas bem-sucedidas na medida em que excederam a taxa de substituição ($\lambda > 1$). A relativa estabilidade demográfica observada na população Montana foi substituída por uma dinâmica acelerada com altas taxas de crescimento e recrutamento na população Submontana, padrões estes que respondem afirmativamente à primeira pergunta deste trabalho sobre a influência da altitude na dinâmical populacional de *B. australis*. Ainda que ambas estratégias tenham sido bem-sucedidas,

essa influência aponta para uma pequena superioridade do desempenho de *B. australis* na área Submontana (λ = 1,084) em relação à Montana (λ = 1,022), resultado que não concorda com o padrão de abundância abservado, mas concorda com a diversidade genética intrapopulacional, tema da quarta e última pergunta deste trabalho. Além da maior riqueza alélica, a população Submontana demonstrou maior quantidade de alelos raros do que a população Montana, sugerindo que o ambiente experimentado nas altitudes maiores provavelmente corresponde a um ambiente mais seletivo, eliminando estes alelos raros. Os resultados observados dão suporte à ideia de que a FOD Montana, situada nas altitudes mais elevadas, abriga condições mais restritivas ao desempenho de *B. australis*, mas que por outro lado, neste ambiente menos competitivo, a relativa estabilidade demográfica observada parece ter sido suficiente para que *B. australis* consiga se perpetuar. Conclui-se então que as estratégias demográficas adotadas em ambas partes do gradiente altitudinal podem ser consideradas bem-sucedidas em seus respectivos contextos ecológicos.

A variação altitudinal encontrada na Serra do Mar pode ser considerada, portanto, como uma barreira para as populações de *B. australis*, criando assim um cenário potencial para a existência de adaptação às diferentes condições impostas pelo gradiente. Notáveis variações fenotípicas já podem ser observadas entre estas populações localizadas nos extremos do gradiente, mas experimentos de transplante recíproco e de jardim comum poderão contribuir para efetivamente responder essas questões.

De maneira geral, a noção de que diferenças altitudinais podem representar barreiras às populações naturais, mesmo num contexto de florestas aparentemente contínuas, é de extrema relevância quando se pretende traçar estratégias de manejo visando à conservação de espécies e genótipos e também de restauração ecológica. Ainda nesse sentido, a constatação de que o ambiente que abriga o maior número de indivíduos não necessariamente corresponde ao ambiente de maior diversidade genética pode também ser de grande utilidade para a execução de tais estratégias conservacionistas. Além disso, pudemos verificar a importância de avaliar a demografia aliada à genética populacional para melhor compreender o contexto em que as populações estão inseridas e com isso avaliar mais acuradamente a dinâmica local. Sendo assim, abordagens que privilegiem a união entre genética e demografia são mais completas e, portanto, preferidas.

Por fim, em um cenário de mudanças climáticas, investigações acerca das respostas das espécies de plantas às diferentes condições ecológicas são de fundamental importância para prever as respostas destes organismos às mudanças ambientais e, nesse sentido, gradientes de altitude são excelentes laboratórios naturais. Nosso estudo revelou que uma espécie de distribuição ampla pode se perpetuar em ambientes climaticamente tão diversos quanto os extremos de um gradiente altitudinal nos trazendo perspectivas menos pessimistas em relação a cenários futuros, ao menos no que concerne a estas espécies dotadas de ampla distribuição e plasticidade demográfica.

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