“Can the native Rusty Fig, *Ficus rubiginosa*, beat climate change?”

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This report is provided to the Australian Flora Foundation in fulfillment of the conditions of the grant awarded to the author in 2015. This report presents the results from a PhD study conducted by K David Mackay between 2014 and 2017 on a keystone, fleshy-fruited tree, *Ficus rubiginosa*, in the western, drier parts of the species' range in northern NSW.

The study culminated in a dissertation submitted in November 2017 for the award of Doctor of Philosophy at the University of New England, Armidale NSW 2351

The ideas, experimental work, results, analyses and conclusions presented in this report are entirely the author's own effort, except where otherwise acknowledged. This work is original.

29th November 2017

Signature of author, K. David Mackay

Date

Cover photo: *Ficus rubiginosa* in typical isolated, rocky-outcrop habitat. Ten mature fig trees grew at this site near Delungra in northern NSW. Photographed 6th May 2014.

Can the native Rusty Fig, *Ficus rubiginosa*, beat climate change?

Abstract

The primary aim of this study was to determine if climate change was impacting on reproductive success in *F. rubiginosa* and/or was likely to impact in the future. *Ficus rubiginosa*, the Rusty Fig, is a widespread, keystone, dry-rainforest species with a 3,000 km range from Cape York, Queensland, to south-eastern NSW. *Ficus rubiginosa* is the only tree on the western side of the Great Dividing Range that produces large quantities of fleshy fruit throughout the year, supporting large numbers of frugivores, insects and insectivores. Field work for this study concentrated in the western half of *F. rubiginosa*'s range in northern New South Wales, Australia, with a smaller number of coastal populations in northern NSW included in the study for comparison with inland populations. Four key areas of population ecology relating to phenology, pollination, avian visitation and genetic variation were addressed in the study. Environmental factors impacting on *F. rubiginosa*'s fitness in these four areas were identified and measured over the three- year period between January 2014 and January 2017. Further field work was conducted over the 3,000 km range of the species in July to October 2015 to collect genetic samples for Next Generation Sequencing and Single Nucleotide Polymorphism (SNP) analysis to determine current and historic patterns of genetic variation across the species' fragmented distribution.

Major findings from this study were that phenology was impacted by seasonal variations in temperature and by irregular, unpredictable variations in rainfall. Low winter temperatures caused syconium and pollinator-wasp development to slow down and go into stasis. Low rainfall stimulated the extension of male and female phenophases in fig trees through staggered syconium development. This staggered syconium development increased reproductive assurance by increasing overlap of male and female phenophases among trees. However, it also reduced the number of ripe fruit on trees at any one time, thereby reducing a tree's attractiveness to frugivore seed- dispersers. Pollination success was reduced in drier parts of *F. rubiginosa*'s range compared with that in more mesic environments. Extreme fragmentation – single trees – provided pollinators with refuge from parasitoids leading to increased male and female fitness in *F. rubiginosa*. Retention of unpollinated fruit on trees increased female fitness by increasing apparent crop size and attracting greater visitation by frugivores. *Ficus rubiginosa* was a pivotal and keystone resource for twice as many insectivorous bird species as frugivore species. Drier climates during the last ice age led to contraction of *F. rubiginosa* populations to four probable refugia, scattered over 22° (2,500 km) of latitude. Dry coastal zones that functioned as

biogeographic barriers to gene flow during the last ice age have functioned as corridors facilitating gene flow between inland and coastal populations more recently.

Signals of early population decline can be difficult to recognise in long-lived species such as large *Ficus* trees. However, several factors that impacted on pollination success and seed production and dispersal in *F. rubiginosa* were identified during this project. Among these factors were fire, rainfall, aridity in intervening habitat matrices, crop size in individual trees and *F. rubiginosa* population size. Fire is projected to keep increasing in frequency and intensity under the influence of climate change. Flower and fruit production were delayed by up to five years and more in many trees across the study area, as trees recovered (resprouted) after fire. Lower rainfall resulted in slower and reduced fruit production and smaller fruit with fewer seeds. Smaller crops of ripe fruit in trees attracted lower avian visitation and, hence, reduced seed dispersal. Lower pollination success was recorded during drier conditions. And gene flow was obstructed by dry areas between populations. All of these factors and events are consequences of deteriorating rainfall and increasing temperatures, which have been experienced across the study area over the last few decades. Further decline is likely and local population extinctions are possible in those parts of *F. rubiginosa*'s range where climate change results in further reductions in rainfall and ongoing increases in temperature. This will negatively impact on the communities *F. rubiginosa* lives in and on ecosystem services provided by the affected plants and animals in these communities within the local and surrounding environments.

Ficus rubiginosa would be an important species to use in restoration as it provides a year-round food resource for frugivores and, as found in this study, a year-round and unique food resource for insectivorous woodland birds, many of which are in decline in this region and others globally.

Introduction

Environmental changes are likely to impact on *Ficus rubiginosa* directly through phenological responses to climatic variation and indirectly through behavioural responses by its pollinators and avian visitors to climatic and habitat alteration. Many of these changes could reduce individual fitness and population viability in this keystone species. Some of these environmental pressures at least, such as habitat fragmentation, fires and drought, are predicted to increase for this species across the region of this study and are likely to become long-term changes under the influence of climate change (CSIRO 2016) and a growing human population (Halpern et al. 2015). Similar environmental changes and stresses are being faced by most species globally (Cochrane 2001, Laurance and Williamson 2001, Cochrane and Laurance 2008).

Four key questions relevant to plant population ecology in fragmented landscapes were identified as relevant to this project. An overview of these four research questions is provided here.

Question 1. What impacts do climatic variations have on flowering and fruiting phenology in *F. rubiginosa* trees growing in a temperate, seasonal environment?

The secondary impacts of climate change on gene-flow in this keystone *Ficus* species via the direct impacts on flowering and fruiting phenology have implications for the future population viability not only in this *Ficus* species but also for other species dependent on it. The effects of climate change such as increasing temperatures or increasingly intense and/or frequent catastrophic climatic events such as cyclones or drought on *Ficus* flowering phenology are unknown. Flowering and fruiting patterns have been investigated in a number of *Ficus* species (Bronstein 1988, Michaloud 1988, Yang et al. 2014, Bianchini et al. 2015) but this is the first study to examine phenological variations in response to climatic variation over multiple years and then relate these changes to pollinator visitation and seed-disperser visitation and the likely ramifications for gene flow under a changing climate.

Question 2. What impacts do climatic variation and fig-population fragmentation have on pollinator dispersal among *F. rubiginosa* trees and, if there are impacts, what are the consequences for pollen dispersal and fruit set?

Studies of fig-pollinator dispersal (e.g., Nason et al. 1996, Yu et al. 2010) have focused primarily on tropical, mesic biomes (but see Ahmed et al. 2009, Gates and Nason 2012). This study contributes to filling a gap in our knowledge by studying fig wasp dispersal in a seasonal, temperate climate which is precisely the climate type that Janzen (1979) suggested may lead to variation in pollinator behaviour and pollen dispersal.

Question 3. What impacts do climatic variation and fig-population fragmentation have on frugivore visitation to *F. rubiginosa* and, if there are impacts, what are the consequences for seed dispersal?

The impacts of habitat fragmentation and climate change on avian frugivores have been found to be significant in other systems, leading to reduced fruit dispersal in many plant species: Cordeiro and Howe (2001) showed that fruit dispersal and seedling recruitment both declined as a consequence of habitat fragmentation and the consequential loss of frugivores. Moran et al. (2009) found the abundance of avian frugivores was reduced significantly in fragmented rainforests in eastern Australia and that dispersal of frugivore-dependent plant species declined as a result. Mokany et al. (2014) show that seed-dispersal services provided by frugivores in rainforests across the wet tropics of northern Australia will be severely and negatively impacted by climate change which may impair the abilities of many rainforest plants to shift their ranges in response to climate change. This is the first study of the current and potential impacts of habitat fragmentation and climate change on frugivore visitation to *F. rubiginosa* and the implications for dispersal by this keystone species.

Question 4. What have the long-term patterns of gene flow and range shifts by *F. rubiginosa* been in eastern Australia?

Genetic and genomic studies have been increasingly used to investigate long-term patterns of dispersal and range extension in plants in response to past climatic changes (e.g., Rossetto et al. 2015) and to model future changes in response to projected climate-change scenarios (Mellick et al. 2012). In this study current patterns of genetic variation across the range of *F. rubiginosa* were identified and past patterns of range extension by the species deduced. Localities of refugia that *F. rubiginosa* populations contracted to in the last glacial maximum were identified and similarities and differences between this species and other species for which similar landscape genetic studies have been conducted are discussed.

The primary aim of this project was to combine the results from these four studies to discuss the likely impacts of future climatic and habitat changes on fitness, dispersal and range-shifts in *F. rubiginosa.* A second but equally important aim was to identify signals of early population decline in local populations of *F. rubiginosa* and to discuss the potential ramifications of decline in this keystone, fleshy-fruited tree in the region.

Methods

Study sites:

This study was conducted primarily in the New England Northwest region of New South Wales (Fig. 4.01), between 29.4 and 31.2° south and between 148.7 and 152.4° east. Additional sites pertinent to particular aspects of this project are described in the relevant sections.

The study area experiences warm to hot summers and cool to mild winters. Average annual rainfall varies from 600 mm to 800 mm west to east (BOM 2017). Altitudes across the study area range between 100 m and 1500 m with *Ficus rubiginosa* populations restricted to altitudes below 1000m (Atlas 2017). This region of eastern Australia has been extensively cleared for agriculture, with over 60% of the woody cover of natural vegetation having been cleared across the study area (Benson et al. 2010). This habitat clearing has left a patchwork of large and small remnants of natural vegetation and single paddock-trees across the landscape. Twenty-four sites were established in the study area in 2014, evenly spread across three habitat-fragment/fig-population size-categories: category 1 = single trees growing within production landscapes or disturbed vegetation (eight sites); category 2 = small populations of between five and fifteen fig trees growing in habitat fragments (eight sites); category 3 = over fifty trees growing within contiguous natural vegetation (eight sites); (Fig. 2, Google Earth satellite images of examples of the three population sizes.)

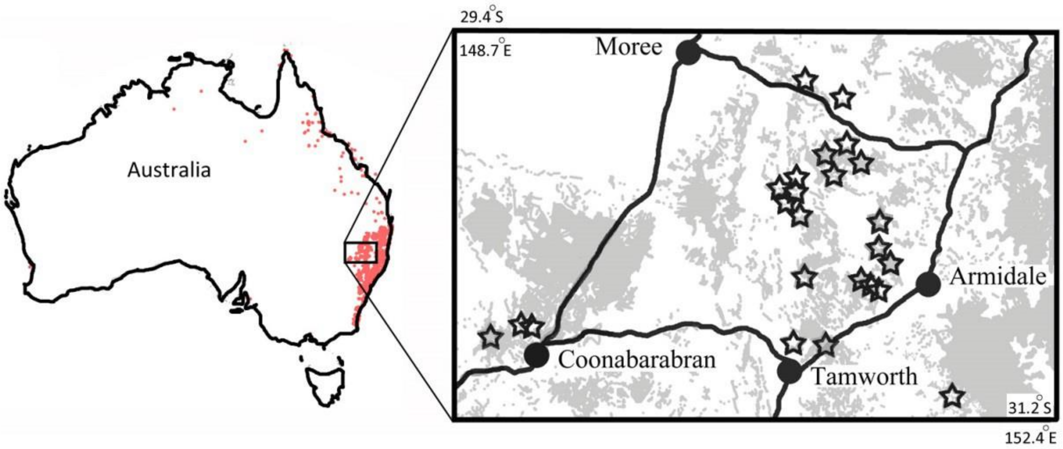

Figure 1. Distribution of *Ficus rubiginosa* in Australia (red dots; includes islands off the Queensland coast), and locations of the 24 study sites (stars) within the 50,000km2 study area in northern New South Wales where this study concentrated. Remaining areas of woody vegetation in the region are marked in grey on the map of the study area, based on satellite imagery (Google_Earth 2017).

Phenology:

Timing and duration of flowering and fruiting events were monitored across 58 trees in 16 sub-populations across the New England Northwest region of northern NSW (Fig. 1) between January 2014 and January 2017. All 58 trees were tagged and georeferenced using a hand- held GPS unit (Garmin Oregon 650). On each monitoring visit, at each tree with syconia, 100 fruits were counted and within these 100 fruits the fruiting stage (A, B, C, D or E, Galil and Eisikowitch 1968, Fig. 3) of each fruit was determined (or the tree was recorded as vegetative if syconia were absent) to measure the proportion of the total fruit on the tree at each stage. Fruit development was correlated against mean monthly rainfall and temperature. Temperature and rainfall variation was recorded for each site through either Bureau of Meteorology records (BOM 2017), landholders' rainfall records or data-logging rain-gauges set up on-site when either of the former two avenues were either unavailable or deemed too distant to be reliable. The region experienced worsening drought conditions from two years before the study began (BOM 2017) until late winter in the third and last year of data collection. The impact of drought was measured as the percentage of trees suffering dieback, with drought duration as a factor (duration determined as the number of months from the beginning of the study until the drought broke in the 31st month of the study).

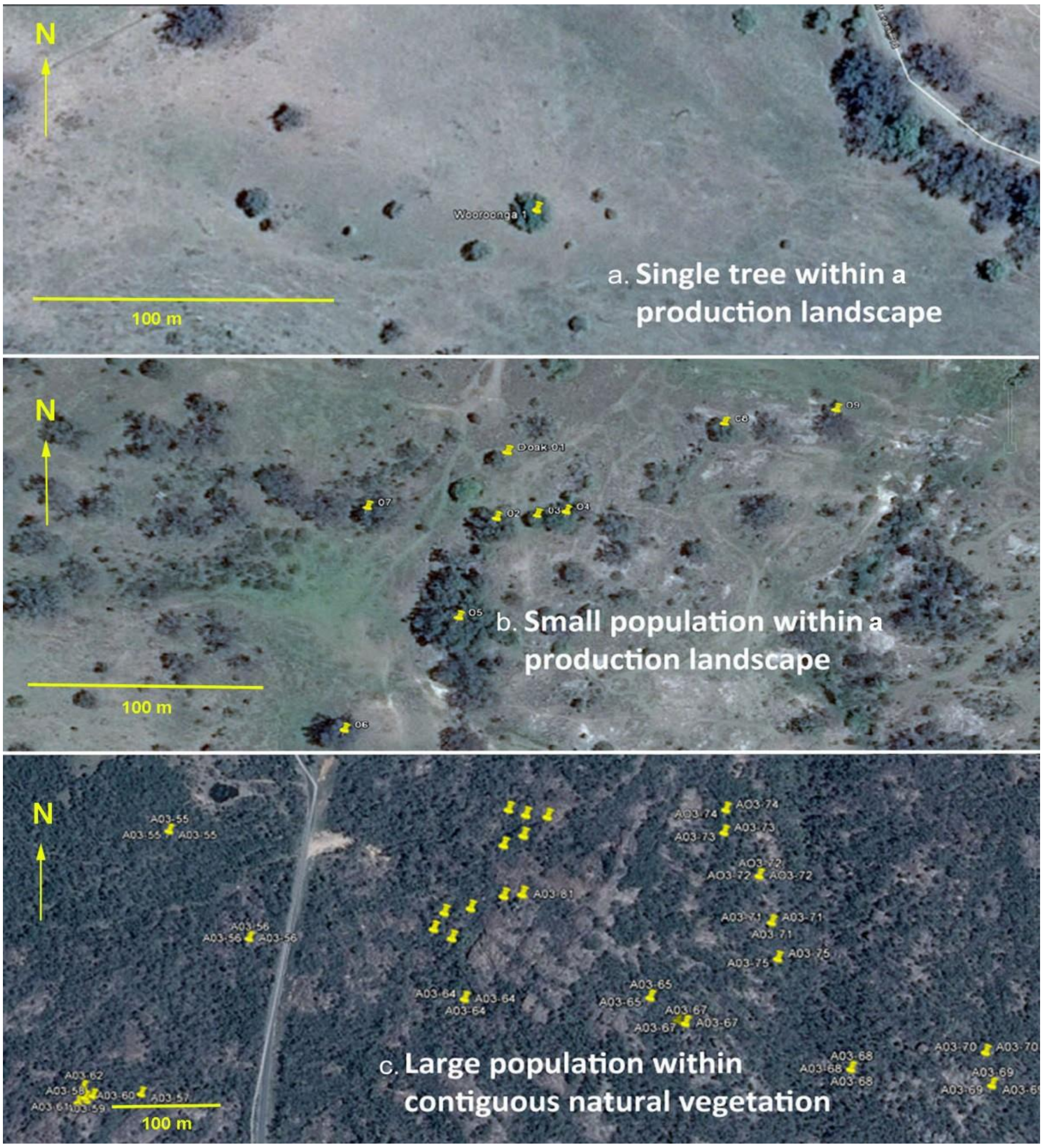

Figure 2. Satellite images (Google_Earth 2017) of the three *Ficus rubiginosa* population-size categories: a) single fig trees within production landscapes (‘Wooroonga 1’ population), b) small populations <16 trees in small habitat fragments surrounded by production landscapes (‘Doak’ population), c) large populations >50 trees in large areas of contiguous natural vegetation (‘Copeton Dam’ population). *F. rubiginosa* trees are marked with yellow pins. Trees not so marked are not fig trees.

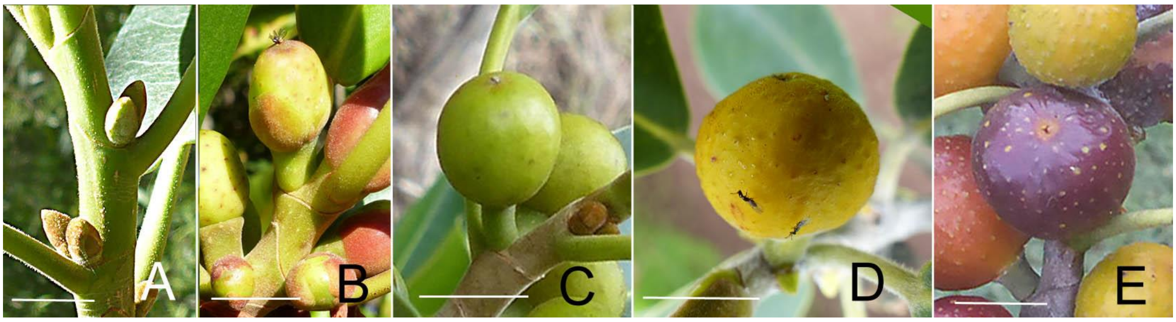

Figure 3. Five recognised stages of syconial development: A = bud stage, B = female stage, C = seed-and-wasp-development stage, D = male stage, E = ripe stage (Galil and Eisikowitch 1968). Scale bars = 10 mm. Images were taken in 2015 at northern NSW sites: A at 'Bereen', B at 'Doak', C at 'Delungra', D at 'Doak' and E at 'Grafton Rd'.

Staggered initiation and receptivity among within-tree syconia is a way a tree can lengthen the female phase of flowering (Smith and Bronstein 1996), and it is this strategy that was observed occurring in *F. rubiginosa*. The likely impact of extending B-stage and D-stage duration on a tree's reproductive success was examined. To do so, overlap in the number of trees with D-stage fruit across 4 observed categories of B-stage duration (1, 2, 3 and 4 months), and overlap in the number of trees with B-stage syconia across seven categories (1 to 7 months duration) of D-stage duration, were compared. Thus, a corollary of the Bronstein et al. (1990) model, that a higher percentage of trees across the study area would have B-stage fruit with receptive female flowers if the duration of D-stage presence on a target tree was extended, was tested. The Bronstein et al. (1990) model was based on field data collected from a population of *F. natalensis* trees in Gabon over a seven-year period from 1974 to 1980 (Michaloud 1988).

Phenology was also measured at the level of individual syconia. The initiation of syconia buds (A-stage syconia) and developmental duration of individual syconia from A to D stage (Fig. 3) was measured by following the development of 5,047 syconia from initiation to ripening: ten branches were tagged on each of 32 trees across 16 sites (43 crops) when buds were initiated on those trees. Subsequent development of each syconium on these branches was monitored.

The study area receives a mean annual rainfall of between 600 and 800 mm (BOM 2017) (mean annual rainfall across the study sites during the study was 728 mm). Rainfall is irregular and unpredictable, generally but not dependently concentrated in the summer months. Temperatures fluctuate seasonally, with winter minima as low as -5°C and summer maxima as high as 45°C (BOM 2017). This region was selected for the study as it contains the highest concentration of *Ficus rubiginosa* populations west of the Great Dividing Range in Australia (Fig. 1).

Results were analysed using graphical methods (Newstrom et al. 1994), analysis of variance (ANOVA) and by fitting general linear models (GLMs) using the statistical package *Statgraphics Plus* version 3.5® and by fitting linear mixed effect models using the lme4 package (Bates et al. 2014) in R (R-Core-Team 2017).

Pollination

Sites: Coastal sites at Sawtell, Urunga, Grafton and Macleay River were included in this pollination study for comparison with results from inland sites.

Wasp numbers: Twenty-two thousand and sixty wasps were collected for analysis from 260 fruit from 80 trees over the 3-year period from January 2014 to January 2017 during increasingly dry, drought conditions. The focus of analysis was to identify patterns of variation among different population sizes in the western, drier parts of *F. rubiginosa's* range.

Pollination success: Total crop size in *Ficus rubiginosa* trees can be limited by the age and size of trees (unpublished data). Comparisons among trees were therefore made at the individual branch level rather than whole-of-tree level. The production of syconia buds (A-stage syconia) and the success or otherwise of their development from A stage to D stage was measured by tagging 10 branches on each of 43 trees across 22 sites/populations and monitoring the development of all individual fruits on those branches. Fruit-set success was measured as percentage of A-stage syconia initially set on a branch that developed into D-stage syconia. Fruit-set success was compared against the degree of flowering asynchrony within crowns to determine if asynchrony increased pollination success. The percentage of fruit that were retained on the tree and continued to develop was used as a surrogate for the percentage pollinated.

Pollination success in *Ficus rubiginosa* was further measured by counting fully developed seeds per syconium. Seeds were counted in the same syconia from which wasps were removed and counted. Results were compared across the three fig population-size categories. The relationship between the mean number of seeds set per fruit and the number of pollinators developing within fruits was tested as was the relationship between the number of seeds set per fruit and fruit volume. Seed-to-flower ratios were compared among population sizes and across the 30-month period of the drought.

Unpollinated fruit retained on trees: In a study of *Ficus pertusa*, Bronstein (1988a) identified a small percentage of fruit retained on trees that had not been pollinated. These were assumed to have been parasitised by wall-galling wasps (Bronstein 1988). A small, exploratory glasshouse experiment was conducted to verify that unpollinated *F. rubiginosa* syconia were able to develop and ripen. Three plants with A-stage syconia developing into B-stage syconia had double-layer bags of fine organza placed over some branches, leaving other branches with developing syconia entirely outside the bags. D-stage syconia were collected from the field and opened D-stage fruit with emerging pollinator and parasitoid wasps were placed within the bags to facilitate pollination of receptive flowers within the bags (Fig. 4). Three other plants with developing A-stage syconia were left untreated. After 7 days the D-stage fruit were removed and bags were removed another 11 days later. The development of each individual syconium was monitored on: (1) branches where pollinators had been introduced, (2) branches of the same plants which had not had pollinators introduced, and (3) on plants which had not had any treatment. All syconia that were retained on the plants and developed to late C/early D stage were collected and dissected under a dissecting microscope to determine if they had been pollinated and/or parasitised. Experimental results from the glasshouse were used to help interpret field observations.

Figure 4. Bagged *Ficus rubiginosa* plant in glasshouse pollination experiment; opened D-stage fruit visible in bottom of organza bag.

Climatic variation: Fruit development in the field was correlated against monthly rainfall. Rainfall data was obtained from Bureau of Meteorology records (BOM 2017), landholders' rainfall records or data-logging rain-gauges set up on-site when either of the former two avenues were either unavailable or deemed too distant to be reliable. Fruit-development success was analysed using Analysis of Variance (ANOVA), and by fitting Linear Models and General Linear Models (GLMs) using the lme4 package (Bates et al. 2014) in R (R-Core-Team 2017). Monthly rainfall variables included in the full models were average monthly rain throughout fruit development, lowest monthly rainfall during fruit development, rainfall in the months before bud-set and fruit ripening, rainfall in the 2 months before bud-set and fruit ripening and rainfall in the 3 months before bud-set and fruit ripening.

Statistical analyses: Data were analysed using ANOVA and GLM functions in the statistical package *Statgraphics Plus* version 3.5®.

Avian visitation

Avian visitors: Five hundred and sixty-two observation periods were conducted at twenty-four sites across the region over three years, from February 2014 to January 2017. Observations were spread across the three *Ficus*-population categories of single trees, small populations and large populations, and across the twelve months of the year. Each observation period was twenty-minutes in duration, considered to be a suitable or minimum survey period to capture bird species diversity and abundance at a tree (Fischer and Lindenmayer 2002). Observations were conducted using binoculars (Barr and Stroud ‘Sahara’ 10x42 binoculars) from the cover of a hide and/or a nearby tree within ten to thirty metres of the observed trees. In each twenty- minute observation period all avian visitors to an individual, tagged tree were recorded. For each individual bird its identification, its activity (eating fruit, eating insects or insect products, or ‘other’ activities) and the length of time spent in the tree over the twenty-minute observation-period was recorded. Variables recorded were latitude, longitude, site, tree, distance from observed tree to nearest non-isolated tree, fig-population size-category, number of trees in the population with ripe or ripening fruit, number of ripe and ripening fruit in the observed tree, date, month, season (3-month seasons of spring, summer, autumn, winter), time of day (Australian Eastern Standard Time), total number of birds, number of frugivore species, total number of frugivores, total time spent in tree by frugivores (summed for all individual frugivores), number of insectivore species, total number of insectivores, total time spent in tree by insectivores (summed for all individual insectivores). All observations were conducted when there was fine, sunny weather with a light breeze. Fourteen twenty-minute observations were conducted in coastal populations of *Ficus rubiginosa* for comparison with inland observations and with results from other studies.

Fruit resources: Trees in which bird observations were recorded were categorised according to the developmental stage/s of fruit in the trees (stages A to E, Galil and Eisikowitch 1968, Fig. 3) or vegetative; and the number of fruit in trees. The total numbers of ripe-and-ripening fruit on trees were estimated by counting 1000 fruit and then extrapolating the area covered by that 1,000 fruit to the whole area of the tree when fruit production was observed to be even around the tree. In cases where fruit production was observed to be uneven over the tree (sometimes fruit production was higher on the northern, sunnier sides of trees) then second and/or further counts were made of 1000 fruit and extrapolated across areas of higher/lower fruit production. Fruit numbers were categorised into 5 logarithmic size-classes to minimise any inaccuracies in fruit-count estimates (categories: 1 = 0 to 20 fruit; 2 = 20 to 200 fruit; 3 = 200 to 2,000 fruit; 4 = 2,000 to 20,000 fruit; 5 = 20,000 to 200,000 fruit).

Insect resources: To assess which insects may be attracting the insectivores, sticky traps (On-Guard outdoor insect catcher from Bunnings Garden Products) were set up at the outer edges of tree canopies about 2 metres above the ground, where much of the insectivore activity was observed, to sample insects within the tree canopies (Fig.5). Three traps spaced at least two metres apart were hung from branches in each of three trees at each of three sites representing the three population-size categories (for the single-tree population-category, three sites with single trees were used). Three traps were set up as controls at one site, with wire cages around them to prevent birds removing trapped insects. Traps were left in the trees for seventeen to twenty-eight days each before being collected. This was repeated three times from September to December 2016. Traps were then photographed at high resolution and all insects caught on the traps were identified to order and counted by enlarging the images on a large 52 x 32.5cm computer screen.

Statistical analyses: Conditional Inference Tree analysis, using the Partykit package (Hothorn and Zeileis 2015) in R (R-Core-Team 2017), was used to assess which of the recorded input variables influenced frugivore and insectivore visitation. Conditional Inference Trees, a recursive partitioning analysis, are especially useful for examining ecological data where input factors may interact hierarchically (De'Ath and Fabricius 2000, Jha and Vandermeer 2010). Conditional Inference Tree analysis is also unbiased, unlike other tree-structured regression models which have a selection bias towards categorical variables with more categories (Hothorn et al. 2006). Factors influencing the total time spent by frugivores and insectivores in trees and species richness were assessed.

Figure 5. Sticky trap set up in tree ('Wooroonga #2') in the Gulf Creek population, 23 September 2016.

Linear mixed effects models (fixed and random effects) were constructed using methods outlined in Winter (2013) in R (R-Core-Team 2017) to analyse the data for frugivore and insectivore visitation to trees. The Likelihood Ratio Test (Winter 2013) was used to attain p-values: saturated or 'full' models, using all measured factors ('effects'), were compared with alternative, 'reduced' models with each reduced model having a single factor removed, using the 'anova' function to determine Chi-square values, degrees of freedom, *p*-values and Akaike's Information Criterion (AIC) values (Burnham and Anderson 2003). The final fitted model accepted contains those factors which result in significant Chi-square value upon removal and a low Δi (difference between AIC values). The following factors were dependent variables in Linear Mixed Effect (LME) analyses: number of frugivore species, total number of frugivores, total time spent in tree by frugivores (summed for all individual frugivores), number of insectivore species, total number of insectivores, total time spent in tree by insectivores (summed for all individual insectivores). The following variables were entered as fixed effects in the Linear Mixed Effect (LME) analyses: latitude, longitude, distance from observed tree to nearest non-isolated tree ('dist'), fig-population size-category ('popsize'), number of trees in the population with ripe or ripening fruit ('numripetrees'), number of ripe and ripening fruit in the observed tree ('numripefruit'), season (3-month seasons of spring, summer, autumn, winter), total number of birds, number of frugivore species ('numfrugspp'), total number of frugivores (‘numfrugs’), total time spent in tree by frugivores (summed for all individual frugivores; ‘frugmins’), number of insectivore species, total number of insectivores, total time spent in tree by insectivores (summed for all individual insectivores). Not all fixed affects were entered in all analyses. For example, for the analysis of factors that affected time spent by frugivores in trees, the number of frugivores and the number of frugivore species were not entered owing to colinearity. The following variables were entered as random effects in the Linear Mixed Effect (LME) analyses: site, tree, date and time of day (Australian Eastern Standard Time). Frugivore- and insectivore-visitation data were heteroscedastic and were log transformed for homoscedacity.

Individual trees (‘tree’) had a significant effect on insectivore visitation but it was suspected that this was a consequence of a tree’s size and hence the number of ripe fruit in that tree. A Kendall rank correlation test (in R) confirmed that ‘tree’ and ‘numripefruit’ were positively, if weakly, correlated (correlation = 0.081, *p* = 0.012) and ‘tree’ was deleted from the model.

Where dependent variables were overdispersed, quasi-poisson General Linear Models were constructed using R (R-Core-Team 2017) for comparison with the outputs from the Linear Mixed Effects models.

A sharp decline observed in insectivore numbers in trees with more than 20,000 fruit, when frugivore numbers peaked, led to further analysis: a linear regression and analysis of variance were performed in R on log-transformed data (loge(1+x)) comparing time spent by insectivores (insecmins) with time spent by frugivores (frugmins) in trees with high fruit loads.

A Chi-square test was used to test the null hypothesis of independence between bird number (frugivores and insectivores) and bioregion (coast Vs inland).

One-way Analyses of Variance were used to assess whether there were any significant differences in the mean numbers of total insects or particular insect groups caught per trap across the five fig-tree fruiting stages: A, in-bud stage; B, female-flowering stage; C, seed- and wasp-developing stage; D, early- ripening/male-flowering/wasp-emerging stage; E, ripe-fruit stage (Galil and Eisikowitch 1968) and F, the vegetative stage between fruiting events. Linear models (Winter 2013) were used to determine which potential factors affected numbers of the pollinator of *F. rubiginosa*, *Pleistodontes imperialis,* in trees.

Landscape genetics

Sites: Sampling design for this project was based on several criteria. Firstly, as this project was in the early descriptive phase of a non-model organism, the design was primarily an exploratory investigation. An attempt was made, therefore, to collect across the species’ range targeting a relatively small number of individuals from each of a large number of populations (localities) based on criteria described in Bragg et al. (2015) and on successful results gained in similar previous studies (e.g., Geraldes et al. 2014). Geraldes et al. (2014) detected complex interactions between climatic variables, isolation by distance and population structure in *Populus trichocarpa* based on 34,000 SNP markers in 498 individuals and Eckert et al. (2010) identified population structure and environmental associations to aridity in the Loblolly Pine (*Pinus taeda*) based on 1,730 SNPs in 682 trees from 54 populations.

Each group of *F. rubiginosa* plants at a site, if separated by at least 1 km from the next *F. rubiginosa* individuals, is referred to as a population in this report. Populations of *F. rubiginosa* varied in size from one to over 100 individuals (personal observation), with most populations having fewer than ten plants. Six samples per population was therefore chosen as a necessary compromise between capturing as much of the variation across the species as well as variation within populations whilst allowing a standard number of collections to be made from every population sampled. Even this number precluded many populations from being included in analyses as there were only one to three individuals in many populations.

Populations of *F. rubiginosa* are concentrated on the coast with areas of significant expansion inland, particularly in northern New South Wales (NSW) but also scattered through central Queensland (Qld) (Fig. 6). Collecting sites for DNA samples were determined using locality data in herbarium records and observations recorded in the Atlas of Living Australia (Atlas 2014) plus on-the- ground searches of potential sites. Populations sampled were scattered across the species' range and included coastal and inland sites, warm tropical to cool temperate sites and mesic to semi-arid environments (Fig. 6). Inland collecting sites were concentrated in northern NSW where the majority of inland populations occur, including the western-most sites in NSW near the Warrumbungles National Park in central-western NSW. Coastal sites were chosen to capture as much of coastal Qld and NSW as possible, particularly on either side of known geographic barriers to plant dispersal (Rossetto et al. 2009, Chapple et al. 2011, Rossetto et al. 2015, Bryant and Krosch 2016) (Fig. 6). Samples were collected in July to October 2015.

Samples: The focus of the sampling strategy was thus to sample as representatively as possible across the distributional range and habitat variation of the species, focusing on number of populations rather than single individuals. One hundred and fifty-four samples were collected from 32 populations in Qld plus 294 samples from 51 populations in NSW. Latitudes and longitudes for each individual were recorded in the field using a hand-held GPS unit (Garmin Oregon 650).

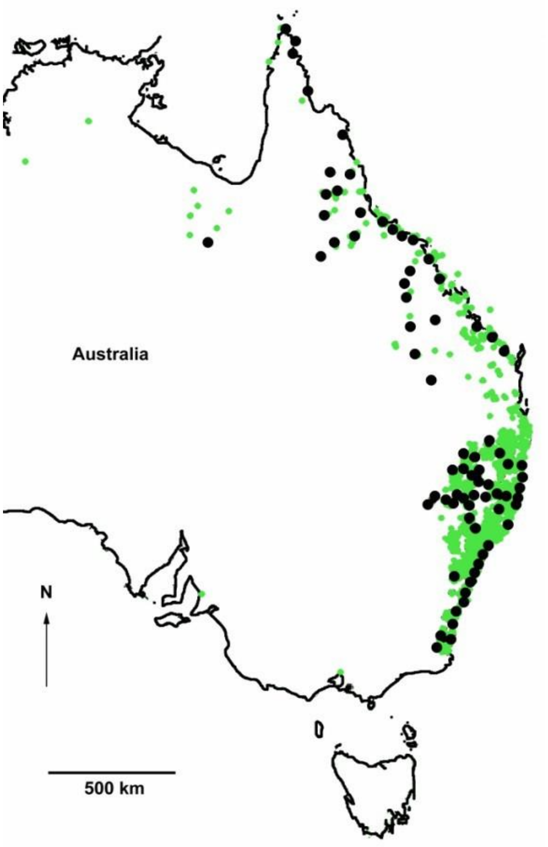

Figure 6. Map of eastern Australia showing localities of herbarium and observation records for *Ficus rubiginosa* (Atlas 2017) (●), and the 83 sites where *Ficus rubiginosa* DNA samples were collected during this study (●).

Each sample comprised a minimum of two fresh leaves (or more if leaves were relatively small) from a single tree. Each sample of leaves was immediately placed in a labeled paper envelope and kept in a field fridge for up to 3 days before being sent fresh by overnight post to the Royal Botanic Gardens Sydney (RBG) where they were placed in an ultra-low temperature freezer before freeze- drying and storage at room temperature prior to DNA extraction. Voucher collections were made at sites where *F. rubiginosa* had not been recorded previously (Atlas 2014). These vouchers are lodged at the Queensland Herbarium at Brisbane.

DNA extraction and sequencing: Samples were sent to Diverse Arrays Technology Pty Ltd (DArT) in Canberra, Australia, for DNA extraction, sub-sampling and sequencing using the proprietary DArTseq platform (Sansaloni et al. 2011). The DArT proprietary process separates low-copy sequences from the repetitive fraction of the genome resulting in the majority of repetitive neutral loci such as simple sequence repeats being removed and subsequent single nucleotide polymorphism (SNP) analyses being concentrated largely on a random sample of coding sequences. DArT sequencing methods employ genome-complexity-reduction techniques using restriction enzymes that allow simultaneous assaying of thousands of markers across a genome (Sansaloni et al. 2011). Sequences were generated using DArT analytical pipelines, producing DArT-generated fastq files of 77bp reads filtered for poor quality sequences and sample barcodes. Approximately 2,500,000 sequences per sample were used in marker calling. Aligned sequences were then collapsed into fastqcoll files which were then corrected for low quality bases using the multiple collapsed tags as a template using another proprietary DArT algorithm. The corrected fastqcoll files were then used in the DArT secondary pipeline for SNP and SilicoDArT (presence/absence of restriction fragments in representation) proprietary calling algorithms (DArTsoft14) (Rutherford, RBG, personal comment). For SNP calling, all tags from all libraries included in the DArTsoft14 analysis were clustered using DArT Pty Ltd's C++ algorithm to facilitate the discrimination of true allelic variants from paralogous sequences. Multiple samples were processed from DNA to allelic calls, using replication and scoring consistency as the main selection criteria for high quality and low error rate markers. Calling quality was ensured by high average read depth per locus of >30 reads per locus.

Quality control was performed on the dataset returned from DArT using the RRtools package developed at Royal Botanic Gardens Sydney (Bragg, unpublished methods). This included removal of six duplicate samples originally included in the DArT analysis for complexity reduction purposes, removal of ten samples that did not produce SNP polymorphism reads and were returned from DArT as 'not reported genotypes', setting a threshold for missing loci at 0.45, removal of fixed loci, removal of poor quality SNPs (with settings at minimum reproducibility = 0.995 and maximum missing data = 0.1) and a random sample of one SNP per clone. After quality control the 384 samples sent to DArT, which came back with over 50,000 unique clones for analysis, were reduced to 364 samples with a little over 16,000 sequences, with single unique SNPs, used for subsequent analyses.

Climate data: Climate data for environmental association analysis was sourced from the Australian Bureau of Meteorology web site (BOM 2017). Data for temperature and rainfall were downloaded on a grid of 0.05° x 0.05° cells, based on the Geodetic Datum of Australia 1994 (GDA 94) coordinate system. Climate variables (averaged over 30 years from 1976-2005) were mean annual rainfall, mean annual temperature, minimum annual temperature, maximum annual temperature, the number of days per annum likely to have frosts below 0°, and the number of days per annum likely to have frosts below minus 2° Centigrade.

Statistical analyses: Initial exploration of data was performed using principal components analysis (PCA) using RRtools (Bragg, unpublished R script). Further data exploration was conducted via PCA and discriminant analysis of principal components (DAPC) using the Adegenet package (Jombart and Ahmed 2011) in R and principal coordinates analyses (PCoA) in GenAlEx (Peakall and Smouse 2006, 2012). Isolation by distance (IBD) was assessed using Mantel tests in GenAlEx (Peakall and Smouse 2006, 2012) to identify correlations between genetic distances (pairwise F_{ST} population distances) and geographic distances (great circle distances between populations) across the species' range and within geographic subsets of the data. Estimations of individual ancestry ('admixture') coefficients were computed using the sNMF function in the LEA package (Frichot and

François 2015) in R. These computations are based on sparse non-negative matrix factorization (sNMF) least-squares optimization (Kim and Park 2011) algorithms (Frichot et al. 2014). Compared with STRUCTURE and ADMIXTURE the sNMF function in LEA does not assume Hardy-Weinberg Equilibrium, is implemented with considerably shorter computational run-times and has been shown to be more accurate and predictive than ADMIXTURE in comparisons using SNP data from the plant *Arabidopsis thaliana* (Frichot et al. 2014). Range Expansion analysis was conducted using the RangeExpansion package version 0.0.0.9000 (Peter and Slatkin 2013, 2015) in R.

The influence of abiotic environmental variables on spatial structure of *F. rubiginosa* populations was assessed using partial Redundancy Analysis (pRDA), an environmental association analysis, using the vegan package (Oksanen et al. 2017) in R (R-Core-Team 2017). The pRDA function in vegan employs a constrained ordination method that partitions variance between niche and neutral processes. Significance of RDA results were obtained using permutation tests (999 permutations), in which the observed relationship is compared with the 999 randomly permuted relationships. If the observed relationship is stronger than the randomly permuted relationships (at $\alpha = 0.05$) then the relationship is deemed significant. Variation partitioning is seen as a useful approach for distinguishing between environmental factors influencing population structure and spatial autocorrelation caused by dispersal (Legendre et al. 2005, Smith and Lundholm 2010).

Results

Phenology:

Bud initiation, female flowering, male flowering and duration of syconia development all responded to rainfall. The duration of crop and individual-syconia development was increased in winter and in response to lower rainfall. A significant decline in female flowering (female phase) was recorded in winter in all three years. A similar decline was observed in male flowering and pollinator dispersal (male phase) in winter but this occurred in only two out of the three years. Male and female phases were extended within crops in response to low rainfall. The extension of the male phase across individual trees led to more trees in male and female phases overlapping temporally (up to 85% of trees overlapping) than the extension of the female phase (up to 44% of trees overlapping). However, extension of phenophases increased reproductive assurance at the cost of there being fewer ripe fruit produced on a tree at a time. In another part of this study, lower fruit numbers resulted in lower frugivore (seed-disperser) visitation. This reproductive assurance strategy is likely to be response to pollinator limitation (34% fruit set in inland populations compared with 84% fruit set in more a mesic, coastal region). Drought conditions led to partial to complete loss of crops to dieback (Fig. 7). These observed phenological responses in *F. rubiginosa* to rainfall have implications for reproductive success and population viability in a predicted climate-change scenario of higher temperatures and lower rainfall across the region.

Figure 7. Loss of fruit and leaves through dieback of branch tips during drought conditions. Photographed at the ‘Strathroy’ population, 50 km north-west of Armidale, in December 2014.

Pollination:

Lower fruit set was observed in drier, inland parts of *F. rubiginosa’s* range compared with that in more mesic, coastal sites. Pollination success measured as fruit (seed)-to-flower ratios declined significantly over a thirty-month period of drought conditions. Thus, drought periods reduce male and female fitness in *F. rubiginosa.* Seeds per syconium and pollinator emergents per syconium were higher in single, isolated trees than in small or large populations. Parasitoid numbers were lower in single, isolated trees. Male and female fitnesses were higher in single, isolated trees than in larger populations of *F. rubiginosa*. Isolated trees thus provide pollinators with shelter from parasitoids (Fig. 8) in a fragmented landscape.

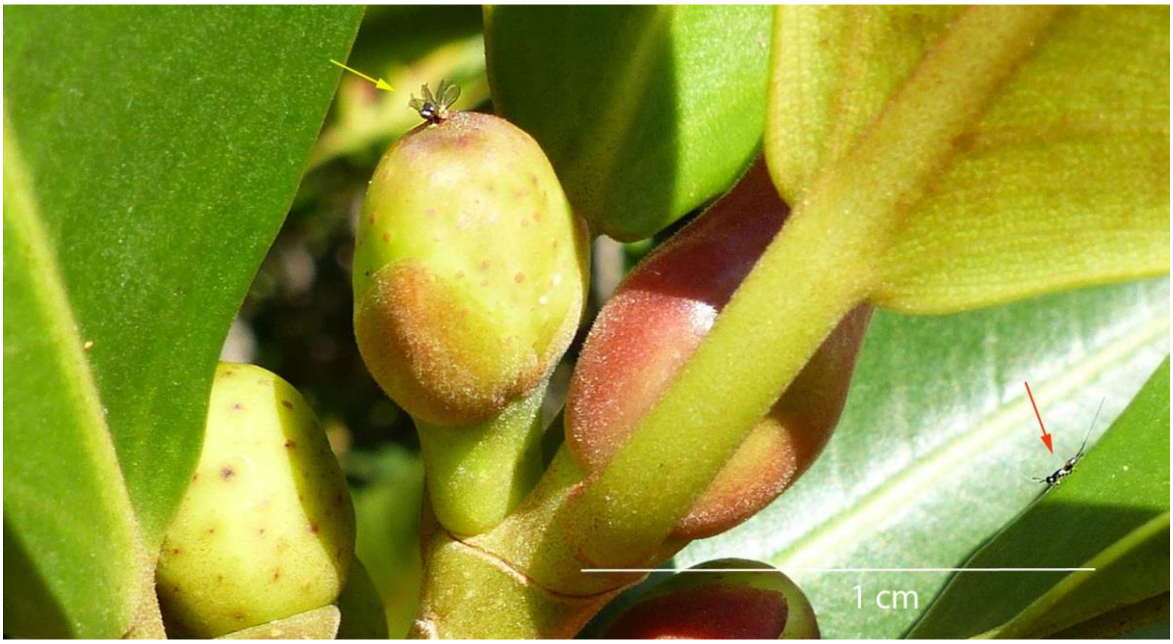

Figure 8. 'B' (female)-stage syconia. B-stage syconia are three to seven millimetres in diameter. A pollinator, *Pleistodontes imperialis*, is entering the ostiole of the syconium at top, and the wings of a pollinator that has already entered the syconium remain sticking out of the ostiole (yellow arrow). Note the parasitoid wasp, *Philotripesis* sp., on the edge of the leaf at centre right (red arrow). Photographed at the 'Doak' site in northern NSW, May 2014.

Avian visitation:

The results from this study shows *Ficus rubiginosa* provides a food resource for over 80 species of birds (Fig. 9) including a more diverse array and larger numbers of insectivorous birds than frugivores. Frugivore and insectivore numbers were influenced by crop size. There is a gap in our knowledge about the importance of these insect-pollinated plants to insectivores which this study goes some way to addressing. Other *Ficus* species are likely to provide similar year-round resources for avian insectivores as well as frugivores throughout tropical, subtropical and temperate regions wherever *Ficus* species occur. Restoring and retaining waning populations of woodland birds is a global challenge that is currently being largely lost. Synergistic drivers of species decline are many including habitat loss and changing climates. Novel as well as established strategies for providing the resources required by woodland birds are going to be needed as species distributions shift and community compositions alter in response to rapidly changing environmental conditions. Increasing numbers of *F. rubiginosa* trees across the landscape could be one effective strategy for supplying year-round food resources for insectivores and frugivores across a fragmented landscape.

Ficus rubiginosa occurs over a large geographical area and a range of environments in eastern Australia, and could be an important umbrella species for conservation of threatened and declining rainforest and woodland birds throughout its range.

Figure 9. Two birds commonly seen in *Ficus rubiginosa*: a Brown Thornbill (left, an insectivore) and a Wompoo Pigeon (right, a frugivore). Brown Thornbill photographed catching insects in *F. rubiginosa* at 'Linden' site, 40 km west of Uralla NSW in December 2016. Wompoo Pigeon photographed consuming fruit at 'Sawtell' site on coast in November 2013 (photo by CL Gross).

Landscape genetics:

This is the first study of genetic variation across the range of *F. rubiginosa*. In this study genetic variation using SNPs and Next-Generation Sequencing techniques, was identified and quantified, followed by analyses aimed at identifying population structure across the species' range. Environmental Association Analysis was then used to identify environmental parameters that were impacting genetic variation in different regions of eastern Australia. Significant population structure was found across *Ficus rubiginosa'*s distribution. A history of range contraction to topographic isolates (Fig. 10) during the Last Glacial Maximum followed by dispersal and range expansion from refugia scattered throughout the species' range were discerned.

Of particular note among the identified ancestral populations were the presences of refugia at the northern and southern extremes of the species' current 2,500 km north-south range, plus two refugia between these geographic extremes. This response by a dry-rainforest species to climate change in the last glaciation is different to that observed in wet-rainforest taxa described in the literature (e.g., Rossetto et al. 2009, Mellick et al. 2012, Heslewood et al. 2014, Rossetto et al. 2015). Differential responses and adaptations to climatic variation were apparent across populations and regions and different survival strategies were detected across identified refugia. Common throughout, however, rainfall and temperature were identified as important drivers of genetic divergence. In spite of strong population structure being a feature of *F. rubiginosa* populations across its geographic range, with clear isolation by distance, geographic clustering of genetic variation and isolation by recognised biogeographic barriers, most of the variation identified in the regional AMOVA (90%) occurred within individuals with five percent of variation occurring among populations and five percent among regions. Limits to *F. rubiginosa's* adaptability and sensitivity to change in these climatic parameters will be important determinants of *F. rubiginosa's* ability to cope with future climate change.

Figure 10. Photo of Wolfgang Peak in central Queensland. This rocky outcrop in otherwise flat country with dry, open, grassy woodland is one of the topographic isolates west of the Great Dividing Range that support populations of *Ficus rubiginosa*. The population of figs on Wolfgang Peak was found growing at the base of the cliffs and in the rocky cleft at the lower right-hand-side of the peak, where water run-off concentrates and accumulates (personal observation). Photographed July 2015.

Discussion

In this study the impacts of habitat fragmentation and climatic variation on fitness in *Ficus rubiginosa* (Moraceae) the Rusty Fig were investigated. There are four key questions relevant to plant population ecology in fragmented landscapes that this study addressed. The first question asked whether flowering and fruiting phenology are impacted by climatic variation. The second question queried the relationships between habitat fragmentation or climatic variation and pollination success. The third question, relating to female fitness in this *Ficus* species, asked whether frugivore visitation and hence seed dispersal were affected by habitat fragmentation or climatic variation. The fourth question asked what the landscape- scale patterns of genetic variation are in *Ficus rubiginosa* and whether there are any environmental associations with these genetic patterns.

These four questions all address the potential impacts of climate change on this species. *Ficus rubiginosa*'s keystone status derives from its critical importance as a food source for many birds that rely on this fleshy-fruited tree west of the Great Dividing Range. Any impacts on this tree's fitness are likely to have flow-on impacts on these other species, on the communities they live in, on their environments, and on the ecosystem services these birds provide in both natural and production landscapes.

Hypothesis of decline: The observations and experimental results presented in this thesis are consistent with a hypothesis that this species is in the early stages of population decline in the western, drier parts of its range. This decline is likely to continue and possibly gather pace if climate-change predictions of lower rainfall, lower humidity, lower soil moisture and increased severity and frequency of droughts and fires (CSIRO 2016) are accurate. This hypothesis is derived from the results of this study and is consistent with the declining population paradigm (Caughley 1994). The reason I feel confident in proposing this hypothesis is because all my results point to declines in fitness in this species in response to declines in rainfall. I explain this further below. Higher temperatures across the region are already occurring, particularly with increases in minimum temperatures in winter and in maximum temperatures in summer (BOM 2017). Increased maxima are likely to cause lower survival and shorter life-spans in the pollinator wasps (Dunn et al. 2008, Jevanandam et al. 2013), leading in turn to increased pollinator limitation and reduced fruit set in the western, hotter and drier parts of the species' range, where fruit set is already less than half that seen in mesic, coastal environments (Chapter 3, Mackay 2017, PhD thesis). This will see *F. rubiginosa*'s range contract eastwards, along with many species that rely on it for a food source. On the other hand, lower winter minima are likely to see the species expand its range into higher altitudes, in common with many other species in temperate zones (Kelly and Goulden 2008, Mellick et al. 2013). *Ficus rubiginosa* is currently restricted to altitudes below 1,000 m in the region, where some peaks are as high as 1,564m (Point Lookout, New England National Park). Pre-adaptation to higher altitudes at some point in the future is not likely to permit *F. rubiginosa* to shift its range immediately. This is a problem faced by many species, such as *Hardenbergia violacea*, which is faced with maximum temperatures increasing above its survival limits in its current range but environmental conditions are not yet suitable in areas where the species could survive in the future (Gross et al. 2017a). Life-spans of 100 years or more in *F. rubiginosa* provide it with an advantage over shorter-lived species in allowing it to ‘wait out’ the progress of climate change and shift its range as other localities become suitable in the future.

Declines in fitness: As mentioned above, observations and experimental results from this study are consistent with declines in fitness in *F. rubiginosa* in response to declines in rainfall. Phenology results (Chapter 2, Mackay 2017, PhD thesis) show that flowering and fruiting phenologies are responsive to irregular rainfall events as well as to regular seasonal change. This is to be expected in a system in which resources (rainfall and soil moisture) are limited and the tight mutualism between plant and pollinator is dependent on asynchronous flowering and fruiting among trees (Janzen 1979). This could change in the future, though, if winter rainfall continues to decline as it has over past decades, currently down 11% since the mid-1990s (CSIRO 2016). Other *Ficus* species occurring in areas exposed to more seasonal rainfall respond with more seasonal leaf and fruit production (e.g., Spencer et al. 1996). Bud production and fruit ripening both follow relatively high rainfall in the two months or month, respectively, before these phenological events in *F. rubiginosa*. Prolonged dry periods cause fruit development in *F. rubiginosa* to go into stasis, as a way of surviving periods of unfavourable weather. If these dry periods become prolonged, as occurred during this study, the duration of fruit development was observed to increase from as short as 3 months under good conditions to as long as 19 months (Chapter 2, Mackay 2017, PhD thesis). Similarly, the duration over which ripening D-stage fruit are produced by a tree and pollinator wasps disperse from these fruit, carrying pollen, can extend for as long as nine months, from as little as less than one month. Periods over which B-stage syconia are produced can extend from less than a month to as long as four months. These phenological variations can act as adaptations to a dry climate and increase the chances of B- and D-stage fruit overlapping among trees so outcrossing can occur. However, with fewer wasps dispersing from D-stage fruit at any one time and fewer B-stage fruit available for pollination the fitness of fig trees is likely to be reduced by the same mechanism by which reproductive assurance is increased. This is the case even if more fruit are produced as fruit production is not going to quadruple or even more. Fruit production per branch is already lower in drier areas than in more mesic environments so further decreases in soil moisture are likely to lead to lower fruit production rather than increased fruit production. This was certainly observed in this study with fewer and smaller fruit produced in response to lower rainfall and whole crops of fruit lost to dieback during drought. Lower fruit production will compound the impact of staggered flowering and fruiting phenology to reduce fitness in *F. rubiginosa* in terms of the number of B- and D-stage syconia present within crowns at any one time. This will mean lower numbers of pollinators emerging within crowns at any one time and reduced pollen dispersal. Lower numbers of ripe fruit on trees to attract frugivores will lead in turn to reduced seed dispersal and lower female fitness. This scenario is consistent with results from my pollination study where I identified average monthly rain during fruit development as having a large and significant impact of fruit set (Chapter 3, Mackay 2017, PhD thesis). Incidents of development stasis and extended development times also reduce the total fruit output by a tree over its lifetime, thereby reducing the tree's life-time fitness. These results are in agreement with those from other studies of *Ficus* phenologies. Bach (2002), in a study of phenological patterns in rainforest trees that included four *Ficus* species, identified rainfall as the primary determinant of leaf flush, flowering and fruiting phenologies in dry-rainforest fleshy-fruited trees in northern Australia. Milton et al (1982), in a 7-year study in Panama, found that even though *Ficus yoponensis* and *F. insipida* trees produced fruit at all times of the year, like *F. rubiginosa* there was an increase in fruit production in response to higher rainfall. The observed phenological correlations with rainfall events in *F. rubiginosa* have important implications for the stability of the plant-pollinator mutualism and the mutualism between the fig and its frugivorous seed-dispersers. Disruptions to these mutualisms could lead to cascades of local extinctions beyond the pollinator and frugivores that would be impacted directly. Species that may be impacted indirectly could include the many insectivorous bird species observed feeding in these fig trees (Chapter 4, Mackay 2017, PhD thesis) as well as the many insect species observed visiting the fig trees during this study. There are also many other plant species in the *F. rubiginosa* dry rainforest communities, which include semi-evergreen vine thickets listed as Endangered Ecological Communities under Commonwealth legislation (Australian_Government 1999), that also depend on these frugivores, insectivores and insects for the ecosystem services they provide.

Disruptions to the fig-pollinator mutualism, either through phenological disruptions or through the direct impacts of climate change, would potentially lead to sharp declines in fitness in *F. rubiginosa.* As with phenology in *F. rubiginosa*, the most important factors impacting on pollination success and fruit set were related to rainfall. Resource limitation was shown to play a direct role in reproductive success in *F. rubiginosa,* with trees in drier areas producing fewer crops, smaller fruit with fewer seeds and fewer fruit per branch than trees with more water resources (Chapter 3, Mackay 2017, PhD thesis). This was observed to be a variable response within populations, though, and highly dependent on microhabitat variation within localities in which trees received the same rainfall but had access to different levels of soil moisture and/or nutrients (unpublished data). Pollinator limitation was identified as a consequence of lower rainfall, with fewer fruit pollinated in dry localities compared with wetter areas or at drier times than wetter times in the same populations (Chapter 3, Mackay 2017, PhD thesis).

When resources (rainfall) are available a percentage of unpollinated fruit were retained on trees in this study, increasing the numbers of ripening fruit on a tree. This was recorded in my phenology study, with unpollinated, small, yellow fruit sometimes remaining on trees after all other fruit had been removed by frugivores. It was also recorded in my pollination study and the possibility that unpollinated fruit could be retained on trees was verified in a small glasshouse experiment (Chapter 3, Mackay 2017, PhD thesis). Visitation rates and fruit removal by frugivores have been positively associated with crop size in other plant-frugivore systems (Davidar and Morton 1986, Saracco et al. 2005, Blendinger and Villegas 2011). Significantly higher visitation rates by frugivores to *F. rubiginosa* trees with larger crops of ripe-and-ripening fruit were found in this study. This supports a hypothesis proposed here: that the retention of unpollinated fruit on trees has been selected by frugivores/seed dispersers which visit trees with larger crops in larger numbers. Thus the retention of fruit that contain neither seeds nor pollinators increases fitness over time in this relatively dry area where pollinator limitation and low frugivore visitation limit seed production and dispersal respectively.

Whilst *F. rubiginosa* displays several adaptations to a dry climate, its ability to cope with more extreme weather is likely to be challenged in the future. Environmental change and degradation are increasing at alarming rates (Pachauri et al. 2014) and the effects are becoming increasingly apparent. Rising temperatures, extreme weather events, coral bleaching, sea-level rises and species extinctions are just some of the more obvious effects of human impacts on the global environment. Three reasons why climate change is predicted to lead to mass extinctions of species globally (Thomas et al. 2004, Urban 2015) are local loss of habitat, limited abilities of species to track shifting climates and the lack of appropriate microhabitats in new, climatically suitable regions. Species' abilities to track or adapt to shifting or changing habitats will therefore determine their future survival or extinction. And our capacity to predict these species' abilities will be pivotal to management and mitigation of biodiversity change and species extinctions. This will be true for *Ficus rubiginosa* as for any species facing decline as a consequence of climate change.

Novel climates are predicted to occur in the future, in tropical and sub-tropical zones (Williams et al. 2007). Habitat changes in these areas cannot be predicted using current, correlative models. It is becoming increasingly apparent that changes to seasonal rainfall regimes as well as temperatures mean that other areas outside the tropics and sub-tropics will experience novel climates as well (Gross et al. 2017a). It is equally apparent from all four aspects of this study – phenology, pollination, avian visitation and genetic variation – that changes to seasonal rainfall regimes will severely impact on fitness and local abundance in this keystone species, just as it has in the past.

Species' range-shifts beyond their known ranges are becoming more frequent consequences of climate change (Davis and Shaw 2001, Walther et al. 2002). The ranges of many species, particularly generalists, have expanded as leading edges of range shifts have advanced faster than trailing edges have retreated (Menendez et al. 2006, Iverson et al. 2011). Whilst *F. rubiginosa* is a specialist rather than a generalist, at least for successful pollination, its range is also likely to increase before it contracts again, simply because of its long life span. Like many other species dispersing to keep up with shifting bioclimatic envelopes, then, *F. rubiginosa* will therefore interact with species in new parts of its shifting range that are adapting to changing conditions rather than dispersing. Many studies of range shifts, in particular regions such as in alpine zones (Pauli et al. 2007) and of individual species or groups of species such as butterflies in the United Kingdom (Menendez et al. 2006), have shown increases in biodiversity rather than decreases. Novel species assemblages are unavoidable consequences of climatic change. However, assisted dispersal of species facing extinction in current localities remains controversial (Ricciardi and Simberloff 2009). Any suggestion of translocating *F. rubiginosa* to novel, climatically suitable areas would require extremely careful scrutiny. Seemingly benign introductions of species into previously uninhabited localities have had negative consequences in the past (Gross et al. 2017b).

The evidence from my genetic study demonstrates that *F. rubiginosa* has undergone significant range contraction and expansion in response to past climate change. There is ample evidence of rapid adaptation and microevolutionary responses to human-induced environmental changes in other species, including niche-shifts (Beaumont et al. 2009), shifts in photoperiod response (Bradshaw and Holzapfel 2001), the rapid evolution of species interactions (Thompson 1998) and morphological changes (Grant and Grant 1993). Adaptability and resilience to change may be higher than assumed in most current predictions of species' range shifts (Rice and Emery 2003, Folke et al. 2004) and may play an important role in keeping extinction rates lower than predicted. However, based on the results of this study of *F. rubiginosa* and its pollinator's current responses to climatic variation, and *F. rubiginosa*'s past responses to climate change identified in my study of genetic variation and environmental association, there is a strong case for stating that *F. rubiginosa* is in the early stages of population decline in the western-most, drier parts of its current range. I suggest that developing a better theoretical framework around the early stages of population decline in *F. rubiginosa*, under the declining population paradigm (Caughley 1994), will help us in building resilience in populations or at least in better understanding and adapting to changes in *F. rubiginosa*'s population dynamics. This will then help us to predict and adapt to the impacts on *F. rubiginosa*'s associated species, including its pollinator (Fig. 11), and communities, including threatened Semi-Evergreen Vine Thicket (Australian_Government 1999). This should help to prevent *F. rubiginosa* from continuing to go into further decline and even becoming threatened with extinction.

Of paramount importance in any strategies aimed at reducing potential extinction rates will be management. Vital management actions will include those aimed at facilitating change such as increasing habitat heterogeneity and successful dispersal or translocations at leading edges of range shifts. Others will be aimed at counteracting the impacts of change such as reducing habitat loss and fragmentation. Resilience to decline in *F. rubiginosa*'s current range can be encouraged through management actions directed towards the species' adaptation to altered environments. Restoration measures are likely to play an increasing role in facilitating range shifts for many species, including *F. rubiginosa*, through the maintenance or provision of suitable habitat for pollinators and frugivores, for example. Facilitation may involve rehabilitation of roadsides, parks, disused farmland and mines, revegetation of degraded or fragmented habitat such as targeted planting of 'future-proof' dry-rainforest vegetation on farms in habitat plantings and windbreaks. Unused rocky outcrops abound within pastured production landscapes in the New England region and lend themselves to plantings of *F. rubiginosa* as the climate at higher altitudes becomes more suitable. Controversial measures including translocations may become necessary tools for managing and preventing species decline. Patch-management practices aimed at accommodating range shifts of incoming species such as *F. rubiginosa* whilst ensuring the survival of extant species unable to disperse (e.g., on mountain tops) may also become increasingly necessary. Enhancing survival through direct human intervention, through planned, science- based management, will be increasingly vital in a world in which negative human impacts on biodiversity are increasing and leading to potential mass extinctions across the globe.

Model limitations Models and hypotheses such as the declining population paradigm can highlight trends through simplification and alert us to issues being faced by populations and suggest possible solutions. Translating theory into effective conservation policy and action, however, has been achieved with mixed success, at least in terms of downgrading the conservation status of many threatened species (Kareiva and Wennergren 1995, Butchart et al. 2010).

Populations of even iconic animals such as tigers and orangutans are continuing to decline in spite of increased public awareness of their plight and millions of dollars having been spent on trying to save them. Not a single species has had its conservation status reduced by modelling alone. More empirical work that tests the application of models is required so we better understand the reality as well as the theory (Laurance 2008).

At a time of increasing biodiversity loss (Wilson 2016) we lack the most basic information on which species exist in most localities. We know even less about species' biology and interactions. In most communities where an immigration credit (Jackson and Sax 2010) is present, for example, information is lacking on the facilitatory role that existing or incoming pollinators or predators may provide to other trophic levels and promote other species' adaptability or resilience to change.

This is true for existing and new habitats both within and outside existing species' ranges. Studies of plant-pollinator mutualisms (Darwin 1862, Bronstein 1994, Gross 1996) and networks (Tilman 1994, Bascompte and Jordano 2007, Olesen et al. 2008), trophic cascades (Fortin et al. 2005) and the impacts of invasive (Gross and Mackay 1998, Gross et al. 2017b) or translocated species (Schwartz et al. 2012, Gross and Mackay 2014), among others, are contributing such information. This study of *F. rubiginosa* and its phenology, pollination, avian visitors and genetics has contributed considerably to our understanding of this keystone species' responses to climatic and habitat change now and into the future.

Ficus rubiginosa displays many examples of resilience to environmental variation including its ability to optimise its phenological response to irregular and unpredictable rainfall events, its ability to resprout after fire and its ability to produce ripe-looking fruit without pollination in order to attract the services of seed dispersers. *Ficus rubiginosa* has endured extreme if slower climate change in the past and is well adapted to a highly fragmented distribution. *Ficus rubiginosa* displays many adaptations to a drying climate including phenotypic plasticity, genetic variation and selection by climatic variables including rainfall, minimum temperatures and maximum temperatures. These adaptations demonstrate *F. rubiginosa*'s resilience to climatic fluctuation. However, limitations to these adaptations point to *F. rubiginosa*'s sensitivity to change in these parameters.

Figure 11.The pollinator of *Ficus rubiginosa*, *Pleistodontes imperialis*, faces an uncertain future in western, drier parts of its range (Photo by CL Gross).

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