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Paper title: Fire, food, and sexual-deception in the neighbourhood of some Australian orchids

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Abstract

The effective use of prescribed fire in biodiversity conservation is currently inhibited by a limited understanding of fire effects on ecosystem processes such as pollination. Orchids inhabiting fire-prone landscapes are likely to be particularly sensitive because they often exhibit highly specialised pollination systems and provide no reward to pollinators, making them dependent on co-flowering heterospecifics to attract and support pollinators. We investigated the hypothesis that fire-driven changes in the local abundance of rewarding heterospecific flowers influence pollination in two rewardless Australian orchid species, *Diuris maculata sensu lato* and *Caladenia tentaculata*. *Diuris maculata s.l.* is thought to achieve pollination by mimicking papilionoid Fabaceae flowers. *Caladenia tentaculata* attracts male thynnine wasps through sexual deceit, and these wasps forage on the open-access flowers of other taxa. We used a space-for-time substitution design with sites in different stages of post-fire succession where we recorded capsule set in *Diuris maculata s.l.*, pollinator visitation to *Caladenia tentaculata*, the floral abundance of rewarding heterospecifics, and abiotic conditions. Many rewarding taxa responded to fire age, but there was only weak evidence that capsule set in *D. maculata s.l.* was positively related to the local floral abundance of rewarding species. There was evidence of an overriding effect of rainfall on capsule set that may have obscured effects of the floral community. Visitation to *C. tentaculata* was not positively associated with any rewarding heterospecifics, and was negatively associated with rewarding *Burchardia umbellata*. Our preliminary findings highlight the need to account for multiple factors when trying to detect fire effects on pollination.

Key-words: sexual deception, food deception, Orchidaceae, fire, pollination

Introduction

Prescribed fire is a widely used tool in biodiversity conservation and land management (e.g. Bond and Van Wilgen 1996; Penman et al. 2011). Substantial knowledge gaps currently limiting its effectiveness in conservation include interactions with ecosystem processes such as herbivory and pollination that influence plant survival and reproduction (Driscoll *et al.* 2010). There is a growing body of evidence from fire-prone regions of North America, South Africa, and the Mediterranean Basin suggesting fire can substantially influence pollination (Bourg *et al.* 2014; Brown *et al.* 2016b; Geerts *et al.* 2012; Ne'eman *et al.* 2000; Pauw 2007; Potts *et al.* 2001; Potts *et al.* 2006; Van Nuland *et al.* 2013). The influence of fire on pollination in other fire-prone regions such as Australia have been poorly studied (although see Brown *et al.* (2016a)).

Orchids inhabiting the fire-prone landscapes of southern Australia and South Africa might especially benefit from fire management of pollination services. These orchids generally form highly specialised relationships with pollinators that make them vulnerable to loss of pollination services (Brundrett 2007; Pauw 2007; Pauw and Bond 2011; Phillips *et al.* 2009; Phillips *et al.* 2014; Phillips *et al.* 2015; Swarts and Dixon 2009). Many are geophytes that respond to hot summer fires with enhanced flowering (Duncan 2012; Lamont and Downes 2011), which has led to the notion that burning (in the appropriate season) to promote flowering and attract pollinators benefits orchids (Coates and Duncan 2009; Coates *et al.* 2006; Cropper and Calder 1990). This was recently supported by Pauw (2007) who found that flowering and pollination of the South African orchid *Pterygodium catholicum* declined as post-fire succession proceeded.

The diversity and complexity of orchid pollination systems may limit the generality of fire management recommendations from studies of individual systems like that of Pauw (2007). *Pterygodium catholicum* rewards its pollinators with oil, such that when fire enhances *P. catholicum* flowering it also enhances pollinator resources (Pauw 2007). However, approximately 6,500 orchid species are rewardless (Jersáková *et al.* 2006), including many fire-stimulated flowering species from the large genera *Diuris*, *Caladenia*, *Thelymitra*, and *Disa* (Beardsell *et al.* 1986; Bernhardt and Burns-Balogh 1986; Dafni and Bernhardt 1990; Dafni and Calder 1987; Indsto *et al.* 2006; Johnson *et al.* 1998; Phillips *et al.* 2009). Rewardless species necessarily depend on rewarding species to support pollinator populations, and may also benefit from enhanced local abundance of pollinators foraging on nearby rewarding plants (the magnet species effect (Laverty 1992)). Rewardless species that mimic rewarding (model) species can also experience enhanced pollination in the presence of the model as pollinators learn to associate rewards with floral traits shared by the model and mimic (Newman *et al.* 2012; Renner 2006). Fire effects on the pollination of rewardless orchids are poorly understood. There is anecdotal evidence that the rewardless Australian orchid *Diuris maculata sensu lato* experiences reduced pollination in the early post-fire environment where it flowers more profusely but the rewarding flowers utilised by its pollinators are scarce (Beardsell *et al.* 1986). There is also evidence that pollinator visitation to the rewardless Australian orchid *Caladenia tentaculata* is enhanced in recently burnt and long unburnt vegetation relative to mid-succession (Brown *et al.* 2016a), though the mechanism underlying this pattern (e.g. fire-driven changes in the local floral community) is unknown.

Here we present preliminary findings from field observations of two Australian orchid species, from the genera *Diuris* and *Caladenia*, as representatives of the two most common strategies used by rewardless orchids to attract pollinators; food-deception and sexual-deception (Jersáková *et al.* 2006). Food-deception and fire-stimulated flowering are thought to be widespread in the Australian genus *Diuris* comprising 60-100 species (Dafni and Bernhardt 1990; Duncan 2012; Indsto *et al.* 2006). Two studies (Beardsell *et al.* 1986; Indsto *et al.* 2006) of *D. maculata s.l.* (including *D. maculata sensu stricto* from New South Wales and *D. pardina* from Victoria) found it to 1) be nectarless (although only *D. maculata s.s.* was tested), 2) share pollinators (*Leioproctus* and *Trichocolletes* bees, and a *Gasterupation* wasp) with rewarding *Daviesia* and *Pultenea* (Fabaceae) species, and 3) share floral morphology (keel flowers) and spectral reflectance (UV nectar guides and similar colour in the bee visual system) with ‘egg and bacon’ peas which are papilionoid Fabaceae species in tribes Mirbelieae (including *Daviesia* and *Pultenea*) and Bossiaeeae. Both studies concluded that rewardless *D. maculata s.l.* (and many other *Diuris* species) mimic the flowers of egg and bacon peas (or Papilionoideae more broadly). Egg and bacon peas often do not commence flowering until three or more years after fire (e.g. Benson 1985; Burrows *et al.* 2008; Knox and Clarke 2004). It is thus possible that *D. maculata s.l.* pollination is influenced by the availability of rewarding pea flowers nearby to support pollinator populations, enhance local pollinator abundance, and/or condition pollinators to associate floral signals with rewards. We investigated the hypothesis that fire-driven change in the local availability of flowers of particular egg and bacon pea species, genera, tribe, or the Papilionoideae sub-family will influence *D. maculata s.l.* pollination.

Sexual-deception and fire-stimulated flowering are common traits among the more than 370 species comprising the Australian genus *Caladenia* (Duncan 2012; Gaskett 2011; Phillips *et* *al.* 2009), including the widespread and common *C. tentaculata* that is the second focus of this paper. These orchids mimic female wasps of the sub-family Thynnidae to exploit the mate-seeking behaviours of male wasps for pollination (Gaskett 2011). The dependence of these wasps on nectar for completion of the life-cycle suggests the importance of rewarding species to sexually-deceptive orchids for pollinator support (Phillips *et al.* 2009). While sexually-deceptive orchids are unlikely to benefit from conditioning of pollinators to associate floral traits with food rewards (i.e. since pollinators visit these orchids for sex not food), magnet species effects are possible though have not been explored. Wasps generally are restricted by short mouthparts to collecting easily accessible nectar (Willmer 2011). Accordingly, thynnine wasps have been observed feeding predominantly on open-access flowers of *Leptospermum, Eucalyptus, Chamelaucium*, *Melaleuca* (Myrtaceae), *Hakea* (Proteaceae), and *Xanthorrhoea* (Xanthorrhoeaceae) (Brown and Phillips 2014). Flowers of these taxa are typically scarce in the early post-fire environment (e.g. Benson 1985; Burrows *et al.* 2008; Enright and Goldblum 1999), though *Xanthorrhoea* and other open-access nectariferous species are abundant (e.g. fire-stimulated flowering *Burchardia umbellata* (Lamont and Downes 2011; Ramsey and Vaughton 2000)) and so may support and attract thynnines while major food sources recover. We investigate the hypothesis that pollinator visitation to *C. tentaculata* is positively associated with fire-driven changes in the local abundance of open-access nectariferous species.

Methodology

Study landscape and site selection

The study landscape is an area of approximately 15,000 km^2 in south-west Victoria, Australia. The area encompasses a number of National Parks and State reserves (including the Grampians National Park) within a predominantly agricultural matrix. The native vegetation is primarily restricted to reserves and consists of sclerophyllous heaths, shrub lands, and woodlands interspersed with open grasslands (Dodson 2001; Gibbons and Downes 1964). The area has a Mediterranean-type climate (hot dry summers and mild wet winters), and has experienced recurring fires throughout the Holocene (reviewed in Dodson 2001). Prescribed fire is applied by land management agencies in an attempt to protect human life and property and achieve ecological objectives (Parks Victoria 2003).

Study sites were selected within the ArcGIS environment according to a number of criteria. 1) Sites were mapped as heathy woodland (Ecological Vegetation Class 48), to control for effects of other native vegetation types with different fire responses and floristics. 2) To enhance independence no two sites could contain areas of vegetation burnt last by the same fire. 3) Sites had to be between 50 m and 150 m from any road to reduce edge effects and enhance accessibility. 4) Collectively sites covered a broad range of time-since-fire (spatial fire history data sourced from Victorian Department of Environment, Land, Water and Planning and ground truthing confirmed mapped fire history was accurate) and so represented a chronosequence (1-75 years, including wild fires and prescribed fires). 5) Sites were not to be burnt by land management agencies during the current prescribed burning season. 6) At least one of the study species or a species from the same genus had to be detected during initial site visits (see data collection section below) to ensure that the vegetation being sampled was representative of the local floral community experienced by geophytic orchids. Under these criteria (and accessibility issues) 41 sites were used.

Data collection

At each site searches were conducted for *C. tentaculata*, *D. maculata s.l.*, and related species between 50 m and 150 m from the road/track until: 1) one or more of either species was detected or 2) 10 mins had elapsed (only after one or more study species had been detected at the site). A 20 x 20 m quadrat was then set up centred on the first orchid detected or at the location where time elapsed. This procedure was continued (while ensuring that quadrats were greater than 40 m apart) until three quadrats had been set up within the site. Up to three flowering individuals (depending on availability) of each species were tagged (using white plastic tags inserted into the ground approximately 5 cm from the flower and labelled with the species and A, B, or C) within quadrats. The flower community was surveyed twice in each quadrat, with 10-15 days between repeat visits, from late September until early November of 2014. During each survey the number of flowering individuals of every native species was recorded (no attempt was made to distinguish ramets from genets), and the number of flowers per individual was estimated for each species by counting and averaging the number of flowers on up to three individuals in each quadrat. Tagged orchids were checked for capsule set during flower community surveys and then once more in December (only one plant per quadrat was used for statistical analysis; plant A if it had not been removed through grazing, plant B if A was removed and plant C if both A and B were removed).

Low rainfall can limit capsule set in *Diuris* species (Indsto *et al.* 2006) and rainfall varied across the landscape over which capsule set was observed. Interpolated rainfall data were thus obtained from the Queensland Government Department of Science, Information Technology and Innovation's Scientific Information for Land Owners database (https://www.longpaddock.qld.gov.au/silo/about.html accessed 15/09/2016) to account for rainfall-driven spatial variation in capsule set during statistical analysis. Eight rainfall variables were calculated: total rainfall (mm) in November 2014, October 2014, September 2014, spring 2014, winter 2014, autumn 2014, the six months prior to sampling, and the 12 months prior to sampling.

Insufficient numbers of *C. tentaculata* individuals were detected during initial site visits so an alternative sampling technique was employed. Observations of visitation to artificially presented sexually-deceptive flowers (baiting) is efficient because the male thynnine wasps deceived into visiting these flowers respond rapidly, peaking within several minutes of presentation (e.g. Peakall 1990; Peakall and Beattie 1996). Ten *C. tentaculata* individuals, each with a single flower, were collected from two, large wild populations. Plants were kept in pots in an attempt to prolong flowering and were used in flower presentations until their flower began to wilt or became damaged. Flowers were presented at the centre of each of the previously established quadrats (see above) within a sub-set of sites (n = 20) including only those that 1) were surveyed on clear days with temperatures exceeding 17°C since thynnine wasp activity is sensitive to these weather conditions (e.g. Bower 2007), and 2) did not contain naturally occurring *C. tentaculata* flowers (to avoid intraspecific interactions between bait and natural flowers). Flowers were presented in groups of four individuals in a consistent spatial pattern of pots clustered tightly together. Presenting multiple individuals simultaneously was an attempt to minimise changes in attractiveness when flowers were replaced (i.e. through an averaging effect), since there is variation among individual sexually-deceptive orchid flowers in attractiveness to pollinators (Peakall and Beattie 1996). Searches within 10 m of each presentation location for flowering conspecifics were made prior to presentation and locations shifted if necessary, because thynnine wasps temporarily learn to avoid the area within approximately 10 m of locations where they have been deceived (Wong *et al.* 2004). Each presentation lasted 10 minutes and the number of times the flowers were contacted by thynnine wasps within each presentation was recorded. Temperature was also recorded during each presentation using a Kestrel 3000 Pocket Weather Meter and mean values used as a statistical control in analysis.

Data analysis

All analysis was conducted in the R statistical environment (R Core Team 2014) using the MuMIn package (Barton 2014) to compare models in an information-theoretic framework (Burnham and Anderson 2002), and followed protocols for assessing spatial autocorrelation and determining the optimal structure for random model components outlined in Zuur *et al.*

(2009). General linear models were used to model fire effects on floral abundance (since values were non-integers; see below) and square root transformations were required to remove heteroscedasticity and normalise error distributions. Generalised Linear Models (GLM) were used to model capsule set in each quadrat as a binary response (1= yes, 0 = no) for *D. maculata s.l.* (only quadrats containing tagged *D. maculata s.l.* plants that flowered and either produced capsules or became desiccated at the end of the monitoring period without producing capsules were used in analysis) and number of visits in each quadrat as the response (negative binomial models were required to account for over-dispersion) for *C. tentaculata*. All candidate model sets are described in Table S1. Models with multiple predictor variables were found to have low levels of multi-collinearity (variance inflation factors < 2). Partial regression plots for multiple regressions were produced using the visreg package (Breheny and Burchett 2012).

Flowering species entered models individually, and in combination (i.e. as a single predictor) at various taxonomic levels under the assumption that pollinators experience combined species equivalently (in a sensory, cognitive, and behavioural sense (e.g. Thomson 1981)). Thus floral variables relevant to *D. maculata s.l.* included: 1) *Dillwynia glaberrima*, 2) *Dillwynia sericeae*, 3) *Dillwynia* species combined, 4) *Pultenaea* species combined, and 5) tribe Mirbelieae (which at the study sites included *Aotus*, *Gompholobium*, and *Daviesia* in addition to *Dillwynia* and *Pultenaea*), 6) tribe Bossiaeeae (which at the study sites was only *Platylobium obtusangulum*), 7) egg and bacon peas (Mirbelieae and Bossiaeeae combined), and 8) sub-family Papilionoideae (egg and bacon peas plus *Kennedia prostrata*). Floral variables relevant to *C. tentaculata* (all open-access, nectariferous species present at study sites) included 1) *Leptospermum myrsinoides* (Myrtaceae), 2) *Burchardia umbellata* (Colchicaceae), 3) *Microseris sp.* (Asteraceae), and 4) these species combined with several *Hakea* (Proteaceae) species and *Xanthorrhoea australis* (Xanthorrheaceae) which occurred at less than 10 sites and so were not modelled individually. (Note that if there was no literature describing nectar production and floral morphology for species detected in the present study it was assumed they were nectarless and so not included in analysis).

While some herbaceous species produced a similar, relatively small number of flowers per individual regardless of fire age, some shrubby species produced numerous flowers per individual that varied with fire age (i.e. as stem growth occurred along post-fire succession). Pollinators therefore may not have experienced individuals of different taxa and/or in different stages of post-fire recovery as equivalent floral sources. Thus two floral abundance measures were compared for each taxon in each quadrat: 1) the number of flowering individuals, and 2) the number of flowering individuals multiplied by the number of flowers per individual. However, results are presented only for the number of flowering individuals as preliminary analyses demonstrated that results did not vary substantially between floral abundance measures 1 and 2 (although the responses of measure 2 to fire were more exaggerated).

The number of individuals of each floral predictor variable was averaged across quadrats (using the maximum of the two surveys for each quadrat) to obtain a single value for each predictor for each site. Between-site variation was then modelled as a function of fire history. The spatial time-since-fire data (see 'Study landscape and site selection' above) were categorised into four age classes which corresponded approximately to heathy woodland growth stages as defined by (Cheal 2010) based on known vegetation fire responses, though with modifications: the two youngest growth stages were rare so we combined them in a single age class, and the oldest age class was rare so it was combined with the second oldest. The age class (AC) categories were thus identified in years since fire as: AC1 (renewal-juvenility) = 0-3 years (n = 13 sites), AC2 (adolescence) = 4-10 years (n = 10 sites), AC3 (maturity) = 11-35 years (n = 12 sites), AC4 (waning-senescence) = 36+ years (n = 6 sites). The same spatial fire history data layer was used to extract for each site the recorded fire frequency (continuous variable with range = 0-5 fires) and minimum inter-fire interval (continuous variable with range = 1-50 years between fire) to be used as predictors since these variables are known to influence population sizes of some of the taxa used in this study (Duff 2010). A separate candidate model set was constructed for each floral variable, consisting of a model containing all single-predictor models, age class and fire frequency, age class and minimum inter-fire interval (the pairwise correlation between fire frequency and minimum inter-fire interval was 0.71 so they were not included in the same model), and a null model with intercept only. These models were compared using AICc (lowest AICc indicates the best model), evidence ratios relative to a null model with intercept only, and R^2, and *p*-values for hypothesis tests were calculated.

The spatially nested sampling design (quadrats within sites) necessitated assessment of spatial autocorrelation. This was performed for each orchid species by inspection of spline correlograms (with 95% pointwise bootstrap confidence intervals) using residuals from the global GLM containing all predictor variables that did not cause multi-collinearity (variance inflation factors <10, see Table S1) with a maximum lag distance of 5,000 m (using the R-package ncf (Bjornstad 2013)). Where spatial autocorrelation was detected, a Generalised Linear Mixed Model (GLMM) with a random effect for site added to the global model was fitted (using R-package glmmADMB for the range of error distributions it allows (Fournier *et al.* 2012)), assessed for spatial autocorrelation as with the GLM, and then compared to the full GLM (i.e. without the random effect) using Akaike Information Criterion for small sample sizes (AICc) and evidence ratios (Akaike weight of GLMM/Akaike weight of GLM).

Next we assessed the importance of rainfall for *D. maculata s.l.* capsule set and temperature for *C. tentaculata* visitation. For capsule set we compared the AICc of eight, single-predictor rainfall models (i.e. one model for each of the eight rainfall variables) to determine the best rainfall predictor, and then calculated the evidence ratio of the best model compared to a null model with intercept only. For visitation we calculated the evidence ratio for a model containing temperature only compared to a null model with a random effect for site (because we detected an effect of site; see 'Results' below). If the model containing the environmental predictor was at least twice as likely as the null to be the best model, and the slope parameter was statistically significantly ($\alpha = 0.05$) different from zero, it was included in all subsequent models as a statistical control and was used as the null model (i.e. model with environmental predictor only) for further comparison.

We then constructed a candidate set of ten *D. maculata s.l.* capsule set models (corresponding to the eight rewarding flower predictors, conspecific abundance, and a null model) and a candidate set of five *C. tentaculata* visitation models (corresponding to the four rewarding flower predictors plus a null model). Models were compared within candidate sets using AICc, explained deviance (D^2), and evidence ratios.

Results

Flowering and fire history

For rewarding species thought to share pollinators with *D. maculata s.l.*, fire history models were better than the null for all floral variables except Bossiaeeae and *D. glaberrima*, and fire history explained a moderate amount of variation (21-42%) (Table 1). The number of flowering individuals for all taxonomic groupings except Bossiaeeae was higher in AC2 and AC3 compared to AC1 (though the differences were not statistically significant for Bossiaeeae, *Pultenea* and *D. glaberrima*), lower in AC4 compared to AC1 (though the difference was not statistically significant for any taxa) (Table 1; Figure 1a). Flowering individuals increased with minimum inter-fire interval for most taxa, though only statistically significantly so for Papilionoideae and *D. sericea*. The number of flowering *D. maculata s.l.*

individuals was highest in AC1 and increased with minimum inter-fire interval, though neither effect was statistically significant.

For rewarding species thought to share pollinators with *C. tentaculata*, fire history models were better than the null for all floral variables except *Microseris sp.* and explained a moderate amount of variation (31-37%) (Table 1). Floral abundance for *L. myrsinoides* and all species combined (dominated by *L. myrsinoides* and *Hakeae* species) was higher in AC2, AC3, and AC4 compared to AC1 (Figure 1b), and increased with fire frequency and minimum inter-fire interval (Table 1), but for *B. umbellata* was lower in AC2 and AC3 compared to AC1 (Figure 1b) and increased with fire frequency but not minimum inter-fire interval (Table 1).

Diuris maculata s.l. capsule set

Spline correlograms did not indicate statistically significant spatial autocorrelation (95% confidence intervals for Moran's *I* statistic overlapped zero at all scales) for *D. maculata s.l.* capsule set (Figure 2a), so no random effect was included. The best rainfall model contained total rainfall during winter 2014, though models containing rainfall during October, autumn, and the previous 6 and 12 months also had substantial support (and pairwise correlations between these predictors ranged from 0.75-0.95). The model containing winter rainfall was 90 times more likely to be the best model (i.e. evidence ratio = 90) compared to the null, the *p*-value for the significance test that the slope parameter equals zero was 0.006, and deviance explained was 34%. The evidence that winter rainfall influenced capsule set justified its inclusion in all subsequent models and use as the null for subsequent comparisons. The best model – and the only model with substantial support – contained winter rainfall plus the number of flowering *D. glaberrima* individuals and had an explained deviance of 52% (Table 2). The evidence ratio for this model compared with the model containing only winter rainfall, which was the third best after the model containing the number of flowering *D. maculata s.l.* individuals, was 7.75. However, the effect of *D. glaberrima* on capsule set was not statistically significantly different from zero (at $\alpha = 0.05$). Capsule set was positively associated with both *D. glaberrima* (Figure 3a) and winter rainfall (Figure 3b).

Caladenia tentaculata

Inspection of spline correlograms revealed significant (95% confidence intervals did not overlap zero) positive autocorrelation at distances less than 500 m, and negative autocorrelation at approximately 3000 and 4500 m (Figure 2b). The inclusion of a random effect for site in the mixed effect model removed this autocorrelation (95% confidence intervals overlapped zero at all scales; Figure 2c), and the full model with this random effect had considerably more support than the full model without this effect (evidence ratio = 65.66). The model containing temperature was less likely to be the best model compared to the null (i.e. intercept plus random effect only) and the *p*-value for the slope test was 0.60, so temperature was not used in further modelling. There was evidence (evidence ratio = 5.91, *p* = 0.04) that the model containing *B. umbellata* was better than the null (D^2 for model with *B. umbellata* as only predictor = 24%), which in turn was better than all other rewarding flower predictor models (Figure 4; Table 2).

Discussion

Flowering and fire history

Flowering papilionoid Fabaceae were generally more abundant at 3-10 years post fire (AC2) and to a lesser extent at 11-35 years (AC3), which is consistent with the literature. The number of flowering individuals was lower at sites with short minimum inter-fire intervals for most taxonomic groupings, which may result from population decline following intervals shorter than the time to first significant post-fire flowering and reproduction (e.g. Keith 1996). The best *D. maculata s.l.* model also contained a positive relationship with minimum inter-fire interval, though this was not significant.

Leptospermum myrsinoides and all open-access flowering species combined exhibited a similar response to Fabaceae taxa but peaked in AC3 as opposed to AC2. The number of flowering *Burchardia umbellata* individuals was higher at recently burnt sites, which is consistent with the literature (Lamont and Downes 2011), and at frequently burnt sites.

Diuris maculata s.l.

Our hypothesis that fire-driven change in the availability of papilionoid (model) flowers would influence *D. maculata s.l.* (mimic) pollination received only weak support. The model of *D. maculata s.l.* capsule set presence/absence as a function of the number of flowering *D. glaberrima* individuals and winter rainfall was substantially better than any other model (including one with winter rainfall only), but the effect of *D. glaberrima* was not statistically significant. Moreover, while *D. glaberrima* displayed the same fire response as most other Fabaceae taxonomic groupings (i.e. peaking in abundance 3-10 years post-fire and declining thereafter), no fire history variable had a statistically significant effect on its floral abundance. Our measure of pollination (presence/absence of capsule set) was relatively coarse and our sample sizes small, such that a more sensitive measure (e.g. proportion of individuals with capsule set) and/or larger sample size may be required to detect effects.

A different sampling design might also improve the chances of detecting effects of fire-driven changes in papilionoid flowers on *D. maculata s.l.* abundance. The spatial scale (grain size) at which rewarding species abundance is measured can determine whether relationships with visitation to co-flowering rewardless species are detected (Johnson *et al.* 2003). In fire-prone landscapes fire age is often heterogeneous at scales corresponding to pollinator flight ranges (e.g. Cane and Neff 2011), such that the fire age (and associated resource levels) of the vegetation patch within which a plant is flowering (as quantified in the present study) is not necessarily the only fire age the animals visiting the plant experience. Given that papilionoid flowers are more abundant in some age classes than others, quantification of the relative frequencies of different age classes within foraging range of *D. maculata s.l.* may more accurately capture the availability of papilionoid flowers to pollinators. Fire age mosaics where pollinators forage on rewarding Papilionoideae in middle age classes and then encounter mostly deceptive orchids in adjacent early age classes is a potential example of the spatial resampling situation that Gigord *et al.* (2002) argue selects for mimicry. Thus while our results provide only weak support for local-scale (e.g. magnet species) effects of fire on pollination through changes in Papilionoideae abundance, our sampling design may have been inadequate to test for landscape-scale fire effects involving pollinator populations dynamics and/or conditioned foraging preferences.

Temporal limitations of our design might also have played a role. Deceptive orchids generally are pollen-limited (Tremblay *et al.* 2005), but we detected a moderate effect of rainfall. This could indicate that capsule set was limited more by resources (water) in the year and/or at the spatial scale of our study, or that wetter areas experienced greater pollinator activity. Though we attempted to account for variation in rainfall statistically, the simple measure we used may have been inadequate. Ultimately, experimental manipulation of soil moisture and pollen-supplementation may be required to disentangle pollen- and resource-limitation. The positive effect of rainfall is interesting in its own right, given predicted declines in growing (winter) and flowering season (spring) rainfall in Victoria (CSIRO and Bureau of Meteorology 2015).

Caladenia tentaculata

Our hypothesis that *C. tentaculata* visitation is positively associated with fire-driven changes in the local abundance of open-access nectariferous species was not supported by the data. We found no effect of the local floral abundance of all nectariferous species combined. We found a negative effect of the local abundance of rewarding *B. umbellata* flowers, which is interesting because while rewarding heterospecifics have been found to decrease pollination of food-deceptive species (Lammi and Kuitunen 1995) we are not aware of similar effects being described for sexually-deceptive species. Our study was correlative so experimentation is required to confirm interspecific competition for pollination, but our results suggest that visitation in the recently burnt environment is not enhanced by changes in the local floral community. It is possible that thynnine feeding resources were incompletely sampled because thynnine wasps are known to consume the sugary secretions of scale insects (Coccoidea and Diaspididae) and lerps (Psyllidae) (Phillips *et al.* 2009) which we did not record. Our results must also be interpreted in light of the fact that while wasp activity will vary through time in response to environmental conditions other than temperature, we observed visitation during a single visit to each quadrat. Quadrats closer in space (within sites) were also closer in time such that the random effect of site we detected may have captured some of this variation.

Conclusions

We did not find strong evidence that in the heathy woodlands of western Victoria fire-driven changes in the local floral community influence capsule set in *D. maculata s.l.* (at least when moisture is limiting) or pollinator visitation to *C. tentaculata*. We stress, however, that our findings are preliminary and pertain to local-scale effects of fire under relatively dry conditions. Trends reported in the present paper warrant further investigation, ideally with experimental control of soil moisture and other conditions.

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Tables

Table 1: shows for each floral variable the best model (lowest AICc), evidence ratio relative to the null, R^2, and parameter estimates (with *p*-value) for 1) the difference in the response between AC1 (the reference category) and AC2, AC3, and AC4 (since they were entered as dummy variables), and 2) fire frequency (FF), and 3) the minimum inter-fire interval (min) if they were included in the best model (bold indicates statistically significant effects at α = 0.05).

Response	Best model	ER null	R²	AC2	AC3	AC4	FF	min
Flower community for D. maculata								
Papilionoideae	AC + min	2.10	0.25	**2.91 (p = 0.01)**	**2.19 (p = 0.04)**	-0.75 (p = 0.60)	NA	**0.05 (p = 0.05)**
egg and bacon	AC + min	2.86	0.26	**3.18 (p = 0.01)**	**2.52 (p = 0.02)**	-0.16 (p = 0.91)	NA	0.05 (p = 0.07)
Mirbelieae	AC + min	28.81	0.34	**3.69 (p = <0.01)**	**2.41 (p = 0.01)**	-0.19 (p = 0.88)	NA	0.04 (p = 0.08)
Bossiaeeae	NULL	NA	NA	NA	NA	NA	NA	NA
Pultenaea	FF	1.02	0.06	NA	NA	NA	0.28 (p = 0.13)	NA
Dillwynia	AC + min	43.00	0.35	**3.29 (p = 0.00)**	**2.00 (p = 0.02)**	-0.13 (p = 0.91)	NA	0.04 (p = 0.06)
D. glaberrima	NULL	NA	NA	NA	NA	NA	NA	NA
D. sericea	AC + min	24.50	0.33	**3.10 (p = <0.01)**	**1.97 (p = 0.02)**	-0.04 (p = 0.97)	NA	**0.04 (p = 0.05)**
D. maculata	min	1.07	0.06	NA	NA	NA	NA	0.05 (p = 0.10)
Flower community for C. tentaculata								
All	AC	3.58	0.36	**17.13 (p = 0.05)**	**25.13 (p = 0.01)**	18.81 (p = 0.07)	**9.00 (p = 0.02)**	**0.60 (p = 0.05)**
L. myrsinoides	AC	43.60	0.37	**23.31 (p = 0.01)**	**27.89 (p = <0.01)**	**25.52 (p = 0.01)**	NA	NA
B. umbellata	AC + FF	19.81	0.31	**-1.73 (p = 0.02)**	**-1.38 (p = 0.04)**	0.00 (p = 0.99)	**0.63 (p = 0.01)**	NA
Microseris sp.	NULL	NA	NA	NA	NA	NA	NA	NA

Table 2: shows for each *D. maculata* capsule set and *C. tentaculata* visitation model the parameter estimate and *p*-value for the floral variable being tested (bold indicates statistically significant effects at α = 0.05), and the change in AICc, Akaike weight, and explained deviance (D^2).

Model	Estimate	*p*-value	Delta AICc	Akaike weight	D²
D. maculata capsule set					
D. glaberrima	0.35	0.16	0.00	0.62	0.52
D. maculata	0.17	0.28	3.90	0.09	0.42
Rainfall (NULL)	0.14	**0.01**	4.04	0.08	0.34
Pultenaea	0.08	0.45	5.57	0.04	0.37
Mirbelieae	0.03	0.42	5.70	0.04	0.37
egg and bacon	0.02	0.40	5.79	0.03	0.36
D. sericea	0.03	0.45	6.09	0.03	0.35
Bossiaeeae	0.02	0.55	6.29	0.03	0.35
Dillwynia	0.02	0.62	6.39	0.03	0.34
Papilionoideae	0.01	0.89	6.62	0.02	0.34
C. tentaculata visitation					
B. umbellata	-0.20	**0.04**	0.00	0.71	0.24
NULL	NA	NA	3.50	0.12	NA
L. myrsinoides	-0.01	0.25	4.44	0.08	0.03
Microseris	0.03	0.64	5.59	0.04	0.02
All	0.00	0.66	5.61	0.04	0.04

Figures

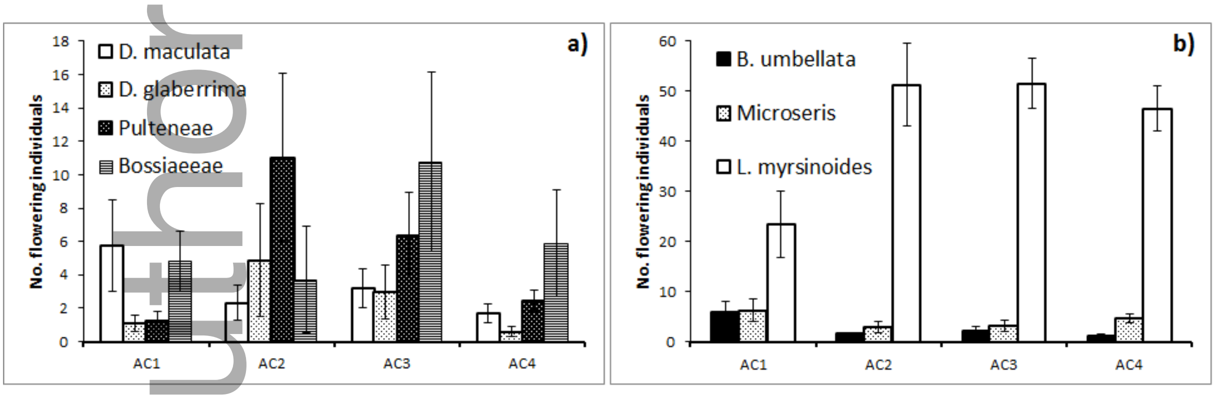

Figure 1: the mean (with standard error) number of flowering individuals of rewarding taxa thought to share pollinators with a) *Diuris maculata* (to enhance clarity only *Dillwynia glaberrima, Pulteneae, and Bossiaeeae* are shown as they demonstrate the range of between-taxa variation in flowering responses to fire), and b) *Caladenia tentaculata* (for clarity the combination of all species is not shown as it is qualitatively similar to the *Leptospermum*

myrsinoides response only larger). (AC1 n = 13 sites, AC2 n = 10 sites, AC3 n = 12, AC4 n = 6 sites).

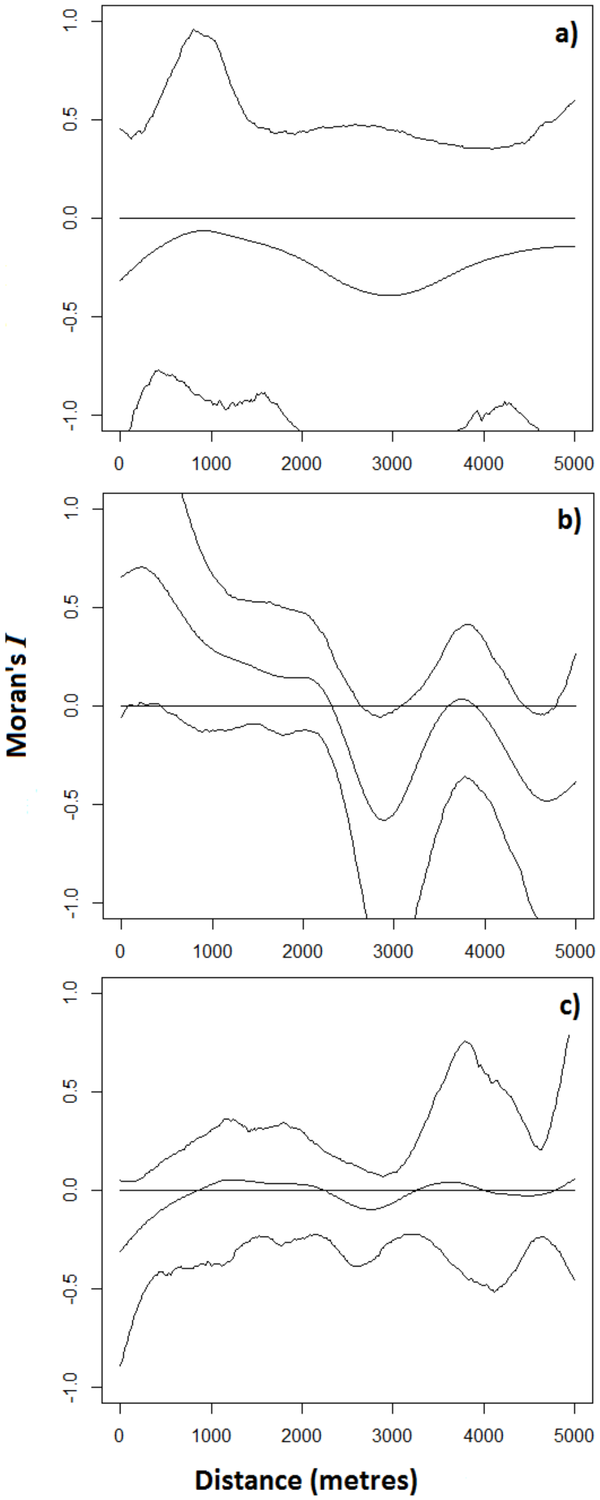

Figure 2: shows spatial autocorrelation (Moran's *I*, with 95% confidence intervals) at a range of distances for the residuals of the a) capsule set model with all (minimally correlated) environmental predictors, b) visitation model with all (minimally correlated) environmental

predictors, and c) visitation model with all environmental predictors plus a random effect for site.

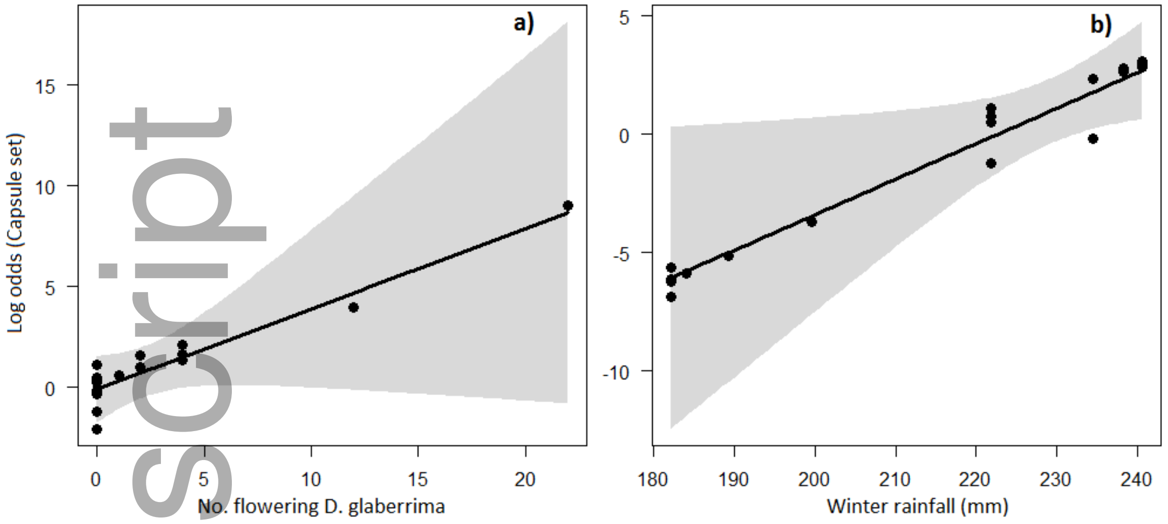

Figure 3: Partial regression plots showing the log odds of *Diuris maculata* capsule set as a function of a) the number of flowering *Dillwynia glaberrima* individuals, and b) winter rainfall.

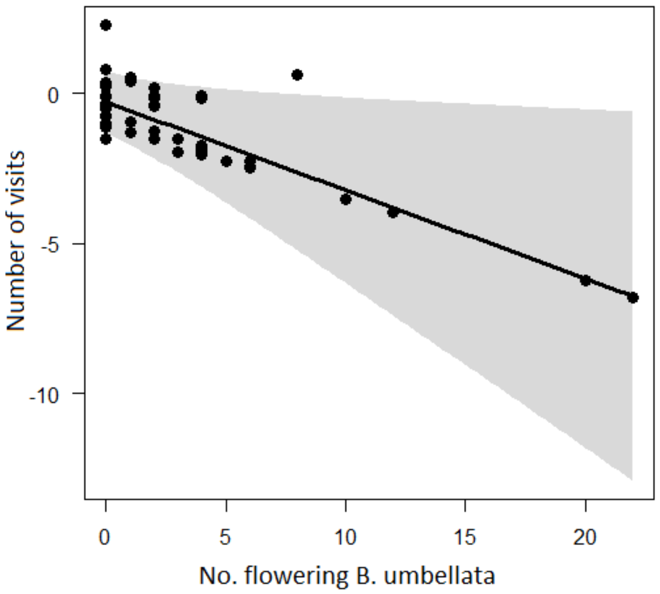

Figure 4: Partial regression plot showing the number of wasp visits to *Caladenia tentaculata* as a function of the number of *Burchardia umbellata* flowers.