



Research article

Fruit colour polymorphism in *Acacia ligulata*: seed and seedling performance, clinal patterns, and chemical variation

KENNETH D. WHITNEY^{1,*} and CAROLYN E. LISTER²

¹Center for Population Biology and Section of Evolution and Ecology, University of California, Davis, One Shields Avenue, Davis, CA 95616 USA; Present address: Department of Biology, Indiana University, Bloomington, Indiana 47405 USA; ²New Zealand Institute for Crop and Food Research, Private Bag 4704, Christchurch, New Zealand
(*author for correspondence: tel.: +1-812-855-9018, fax: +1-812-855-6705, e-mail: kdwhitne@indiana.edu)

Received 15 July 2003; accepted 17 January 2004

Co-ordinate editor. J.F. Stuffer

Abstract. Fruit colour polymorphisms are widespread in nature, but their ecological and evolutionary dynamics remain poorly understood. Here we examine *Acacia ligulata*, a shrub of the Australian arid zone which exhibits a red/orange/yellow aril colour polymorphism. We asked whether the polymorphism had a genetic basis; whether selection acted differentially on morphs during the seed and seedling stages; whether geographic variation in morph frequencies was correlated with environmental factors; and whether morphs differed in physical or chemical characteristics that might influence selection on them. When grown to maturity in a common greenhouse environment, maternal families of seeds showed phenotypic patterns consistent with biparental genetic control of the polymorphism. In contrast to other fruit-colour polymorphic species, progeny of *A. ligulata* morphs did not vary in rates of seedling emergence or survival in a common garden. Sampling along a 580 km transect revealed clinal variation in morph frequencies. Frequencies of the yellow morph decreased, and frequencies of the red morph increased, across a gradient of decreasing temperature and increasing rainfall. Morphs did not differ in seed mass, aril mass, or in profiles of fatty acids and flavonoids in either arils or seeds. However, morphs showed consistent differences in carotenoid profiles and elemental content of arils, suggesting that selection by avian and insect seed dispersers, seed predators and herbivores should be investigated. These patterns indicate that both abiotic and biotic factors may contribute to selection on the *A. ligulata* polymorphism.

Key words: carotenoids, cline, seed germination, seedling survival

Introduction

The dynamics of genetic polymorphisms in natural populations have long been a central focus of evolutionary ecology (e.g., Epling and Dobzhansky, 1942; Sheppard, 1951). Polymorphisms may be selectively neutral, or may be maintained by several selective mechanisms, including overdominance,

frequency-dependent selection, and variable (also known as fluctuating) selection (Gillespie, 1998). Polymorphism thus provides a window on the maintenance of genetic diversity in nature.

Fruit colour polymorphisms are a widespread phenomenon, occurring in at least 19 plant families (Forde, 1986; Willson, 1986; Willson *et al.*, 1989). Fruit colour differences are simply inherited in many plants (Willson *et al.*, 1989), and can be traced to variation in pigments such as carotenoids, anthocyanins, and betalains (e.g., Willson and O'Dowd, 1989). However, the dynamics of selection on fruit colour polymorphisms remain poorly understood for two reasons. First, the leading hypothesis for the maintenance of these polymorphisms has received little empirical support. Seed dispersal agents have historically been viewed as the most likely agents of selection on fruit colour (Willson and Whelan, 1990). While disperser preferences among colour morphs have been found in laboratory and field tests (Willson and Comet, 1993; Willson, 1994; Puckey *et al.*, 1996; Willson and O'Dowd, 1989; Traveset and Willson, 1998), these preferences often do not translate into differences in fruit consumption rates in the field (Willson and O'Dowd 1989; Traveset and Willson, 1998; Traveset *et al.*, 2001; but see Gervais *et al.*, 1999). Consistent with this pattern, nutrient analyses have thus far not revealed variation among morphs (Willson and O'Dowd, 1989; Traveset and Willson, 1998; Traveset *et al.*, 2001). Second, pleiotropic effects of colour alleles may influence the early life history stages of progeny in unexpected ways. Pleiotropic effects of colour alleles on grain dormancy have been demonstrated in wheat (Groos *et al.*, 2002), and studies of two wild, fleshy-fruited species have demonstrated differences in seed germination rates among colour morphs (Willson and O'Dowd, 1989; Gervais *et al.*, 1998; Traveset and Willson, 1998). These patterns suggest that fruiting plants remain a rich area in which to explore the ecological and evolutionary implications of polymorphism.

In this study, we examine *Acacia ligulata* A. Cunn. ex Benth. (Fabaceae), a shrub of the Australian arid zone which exhibits a red/orange/yellow aril colour polymorphism. Separate evaluations of vertebrate and invertebrate frugivores and granivores as selective agents on fruit colour are in progress. Here, we establish a context for these interactions by addressing the following questions: (1) Does the colour polymorphism appear to have a genetic (as opposed to environmental) basis? (2) Does selection act differentially on morphs during the early life history stages of their progeny (seedling emergence and survival), reflecting potentially pleiotropic effects of colour alleles? (3) Given that clinal variation can provide clues about selective agents, is there evidence for variation in morph frequencies on a regional scale? (4) Do morphs vary in diaspore size, pigment chemistry, or elemental chemistry

in ways that might influence selection by biotic agents and/or abiotic factors?

Methods

Study species and study areas

Acacia ligulata is a widespread dune shrub of arid and semi-arid Australia (Maslin and Hopper, 1982) reaching to 5 m in height. Based on marked plants, mean adult longevity exceeds 13 years (T. Auld, pers. comm.). Seed banks are also apparently long-lived, as seed viability did not decline dramatically during a 2-year burial experiment in the field (Auld, 1995a). Initial seed dormancy is high, ranging from 88 to 99% in four populations studied (Auld, 1995a; Letnic *et al.*, 2000). Germination is episodic following rains, and recruitment can be severely reduced by vertebrate herbivores and drought (Auld, 1995b; Auld and Denham, 2001). Flowering can occur within 3 years of germination (Auld, 1995b).

Diaspores of *A. ligulata* are comprised of a seed (ca. 5 mm in length) and an expanded, coloured funicle (the aril or elaiosome). Arils are relatively lipid-rich (50–57% of dry weight) and carbohydrate-poor (9–10%), with moderate amounts of protein (17–22%) (Davidson and Morton, 1984; O’Dowd and Gill, 1986). Maturing seeds are attacked by a range of insect predators, including heteropterans, weevils, and chalcid wasps (Whitney, 2003). Mature diaspores are displayed on the open valves of dehiscent fruits, and are dispersed by a variety of ants and birds (Davidson and Morton, 1984; Forde, 1986; Letnic *et al.*, 2000; Whitney, 2002).

Aril colour is polymorphic with mature shrubs producing either red, yellow, or orange diaspores (O’Dowd and Gill, 1986). A previous report (Davidson and Morton, 1984) suggested that a Northern Territory population of *A. ligulata* with orange arils exhibited an ant-dispersed diaspore morphology, while New South Wales populations of mixed red and yellow morphs exhibited bird-dispersed morphologies. However, the Northern Territory population was misidentified and is in fact *A. bivenosa* (Chapman and Maslin, 1992). Thus, at present there are no data suggesting that *A. ligulata* colour morphs are differentially specialized for particular dispersers. Colour morphs grow in close proximity and are usually distributed at random within populations (unpubl. data), indicating that specialization of morphs to particular microenvironments is unlikely.

Geographic variation in morph frequencies was examined in 13 populations along a 580 km transect in western New South Wales, Australia (Fig. 1,

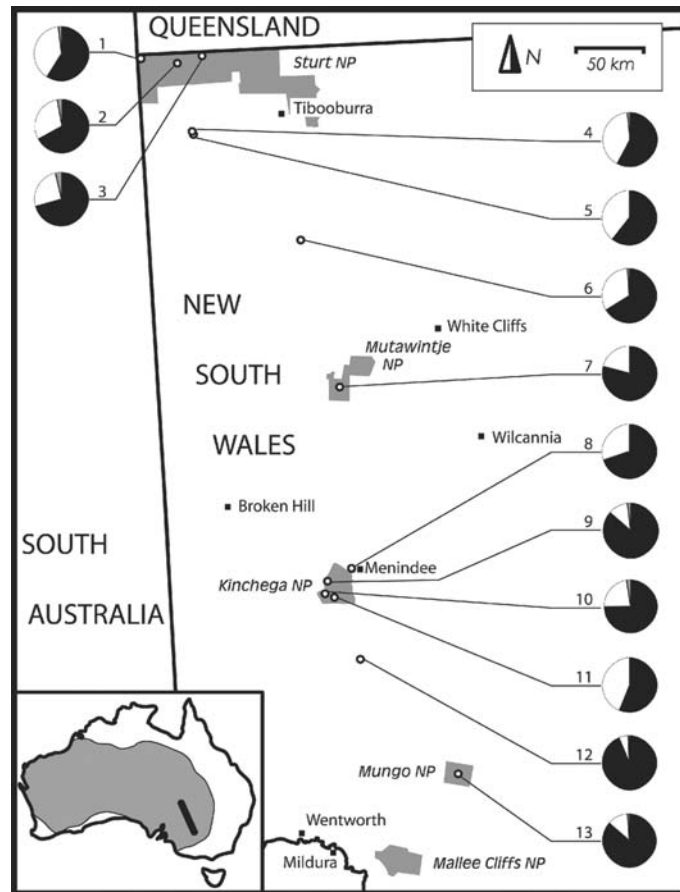


Figure 1. Locations of sampled *A. ligulata* populations along a 580 km transect in western New South Wales. Pie charts reflect frequencies of morphs: red morphs are in black, orange morphs are in gray, and yellow morphs are in white. Inset: location of the transect (solid line) within the range of *A. ligulata* (shaded area).

Table 1). Life history correlates of the polymorphism were examined in three of these populations (Menindee, Cawndilla and Big Dune) within Kinchega National Park (32°30'S, 142°16'E), as described below. In Kinchega, *A. ligulata* flowers in September, and fruit dehiscence and seed dispersal occur from mid-December through February.

Genetic basis of the polymorphism

In an effort to evaluate whether colour variation in *A. ligulata* has a genetic basis, we grew plants to maturity from seed and assessed their diaspore phenotypes in a common greenhouse environment. In December 1998 seeds were

Table 1. Location, physical characteristics, and *Acacia ligulata* morph frequencies at sampling sites in western New South Wales

No.	Population name	Latitude	Longitude	Morph frequencies (%)			Rainfall (mm) ^a	Max. temp. (°C) ^b	Landform	Year sampled
				Red	Yellow	Orange				
1	Sturt NP – Cameron's Corner	29°00'09"S	141°00'05"E	59.0	39.0	2.0	181.6		Dune	2001–2002
2	Sturt NP – Fort Grey Basin	29°04'41"S	141°13'58"E	67.0	30.4	2.7	181.6		Lunette	2001–2002
3	Sturt NP – Camp David	29°01'34"S	141°25'35"E	70.6	25.7	3.7	173.9		Dune	2001–2002
4	Tilcha Creek	29°30'08"S	141°18'33"E	57.8	40.5	1.7	170.6	27.3	Floodplain	2001–2002
5	Hewart Downs	29°31'00"S	141°19'54"E	60.6	39.4	0.0	170.6		Dune	2001–2002
6	Packsaddle	30°15'57"S	142°04'04"E	66.0	33.0	1.0	198.6	26.8	Dune	2001–2002
7	Mutawintje NP	31°14'41"S	142°17'02"E	79.3	20.7	0.0	87	227.7	Sandplain	1999–2000
8	Kincheha NP – Menindee	32°22'48"S	142°23'30"E	69.8	30.2	0.0	106	243.5	Lunette	2001–2002
9	Kincheha NP – Cawndilla	32°26'36"S	142°11'14"E	86.4	11.7	1.9	103	243.5	Dune	2001–2002
10	Kincheha NP – Big Dune	32°32'05"S	142°09'41"E	74.8	22.7	2.5	119	230.7	Dune	2001–2002
11	Kincheha NP – Channel Rd.	32°33'11"S	142°13'00"E	56.2	43.8	0.0	89	230.7	Floodplain	1998–1999
12	Shire Boundary	32°58'57"S	142°24'06"E	93.5	5.6	0.9	107	245.8	Dune	2000–2001
13	Mungo NP	33°44'47"S	143°07'46"E	86.2	12.6	1.1	87	268.9	Lunette	2000–2001
			Means	71.3	27.3	1.3				

^cNP = National Park; *n* = number of adult plants sampled.

^a Estimated mean annual rainfall from nearby BOM recording stations.

^b Estimated mean daily maximum temperature from nearby BOM recording stations.

collected from randomly chosen open-pollinated plants (Menindee and Big Dune populations) and the maternal aril colour recorded. Seeds from each of 11 maternal families were planted in the greenhouse in Davis, CA, USA in April 1999, after dormancy was broken with a 1-min boiling treatment. Plants were grown in modified UC Mix in 3.8 l pots and were watered with a solution of distilled water and fertilizer (GrowMore 4-18-38 No Boron, National Research & Chemical Company, Gardena, CA, USA). Pots were frequently rotated. By spring of 2001, 41 of the 164 seedlings had reached maturity. Plants were hand-pollinated with mixed pollen from randomly chosen donors, and aril colour was assessed at fruit maturity. After pooling families by aril colour, the independence of maternal and progeny aril colour was tested using Fisher's exact test (Proc FREQ, SAS Institute, 2000); orange families were excluded because expected values for some cells were low. Seeds from controlled crosses were also planted, but failed to produce mature shrubs within the period of this study. Accordingly, we could not test for Mendelian inheritance of the trait.

Seedling emergence and survival

If pleiotropic effects of colour alleles on early life history stages exist and lead to selective differences among morphs, we might expect to find them prominently expressed in sites where morph frequencies are highly skewed. Thus, we examined the effects of maternal aril colour on seedling emergence and survival in a field plot located within the Cawndilla population. With red morphs at a frequency of 86.4%, this is one of the most skewed *A. ligulata* populations sampled (Fig. 1, Table 1). The 6 × 4.3 m plot was fenced to exclude vertebrate herbivores such as kangaroos, goats, and rabbits. Seeds from 73 maternal plants distributed among three Kinchegea populations (Menindee, Cawndilla and Big Dune) were collected in January 2000. These collections represented 30 red and 30 yellow morphs (10 of each colour from each population), but only 13 orange morphs (1 from Menindee, 9 from Big Dune, 3 from Cawndilla) because of their natural rarity. Six seeds from each family were boiled for 1 min to break dormancy and were kept in moist petri dishes for 4 days to allow imbibition. On 8 November 2000, seeds were planted in the field plot at 15 cm spacing in a randomized design. Additional 'buffer' seeds were planted at the periphery of the plot to minimize edge effects. As germination from natural *A. ligulata* seedbanks is aseasonal and follows rainfall events (Auld, 1995b), the plot was watered by hand (2.1 cm/week) to simulate a high-rainfall germination period. Watering ceased at 15 weeks. A seedling was scored as emerging if it cracked the soil surface. Seedling emergence was examined every 2 days following planting (excepting day 4); all emergence was completed by day 16. Seedling survival was monitored approximately weekly during the first three months, and at 4.5, 11, and 14 months.

First, the effects of maternal aril colour, population, and their interaction on seedling emergence rates (the cumulative fraction emerged at each census) were examined using repeated-measures MANOVA (SAS Institute, 2000). Population was included in the model only to reduce unexplained variation; it was specified as a fixed factor in order to increase the power to detect an effect of maternal aril colour. Thus, conclusions are applicable only to the sites examined here. Second, the effects of maternal aril colour, population, and their interaction on seedling survival were examined using a proportional hazards model (Proc PHREG; SAS Institute, 2000). Mean survival times of maternal families ($n = 73$, with a mean of 5.2 seedlings per family) constituted the response variable. Seedlings that died before emerging were excluded from this analysis. Average seed mass of maternal families was used as a covariate, as average seed mass did not differ among morphs (see Results).

Clinal variation in morph frequencies

To evaluate variation in morph frequencies on a regional scale, we examined 13 *A. ligulata* populations along a rough NNW-SSE transect in western New South Wales. The 580 km transect spanned a temperature and rainfall gradient, with hotter and drier conditions prevailing in the northwest. Different populations were sampled in different years (1998–2001), but samples were expected to be comparable, as morph frequencies among reproductive individuals changed less than 4% over this period in three well-studied Kinchega populations (unpubl. data). For each population, approximately 100 adults were sampled to determine morph colour, latitude and longitude were determined with a handheld GPS unit, and landform was classified as ‘dune’ or ‘other’ (comprising lunette, floodplain, and sandplain). Mean annual rainfall was estimated for each population from Australian Bureau of Meteorology (BOM) records, using data from up to three nearby stations. Mean maximum temperature was estimated for populations near BOM temperature stations (5 of the 13 populations).

Latitude, longitude, mean annual rainfall, and mean maximum temperature were all highly correlated (see Results); therefore, latitude was chosen as a surrogate predictor variable to avoid problems of multicollinearity (Philippi, 1993). The effects of latitude (a covariate) and landform (both fixed factors) and their interaction on the angular-transformed proportions of the populations that were yellow and orange were tested using MANCOVA (SAS Institute, 2000). The proportion red was not included in the model since it is not independent of the other response variables; because the proportions of red and yellow morphs are tightly correlated (Pearson’s $r = -0.996$, $p < 0.0001$), the model is still informative about the effects of latitude and landform on the frequencies of the red morph. Following significant results in the MANCOVA, protected ANOVAs (Scheiner, 1993) were performed on individual response variables.

Diaspore morphology

Diaspores from 73 maternal plants were derived from the collections for the seedling emergence study (above). For each plant, mean fresh mass per seed and mean fresh mass per aril were determined by weighing a pooled sample of 10 diaspores (Acculab PP-2060D balance, Newtown, Pennsylvania, USA). The effects of maternal aril colour (a fixed effect) and population (a random effect) on seed mass and aril mass were examined with MANOVA (SAS Institute, 2000). Because of the unbalanced sample sizes in the mixed model, least-squares means are presented and standard errors were computed using Proc MIXED (SAS Institute, 2000).

Nutrient and pigment chemistry of diaspores

We examined variation among morphs for three suites of chemical characters: elements, fatty acids, and pigments. Elemental, fatty acid and β -carotene (carotenoid) analyses provide information on potential differences in nutritional quality, whereas pigment analyses examine the visible cues potentially used by frugivores. Pigment analyses were restricted to carotenoids and flavonoids, since betalains (the remaining major class of fruit pigments) are found only in a small clade of plant families not including the Fabaceae (Willson and Whelan, 1990). All plant tissues (seeds and arils) were collected from Kinchega National Park (Menindee, Cawndilla and Big Dune populations). Elemental, fatty acid, and carotenoid samples ($n = 7, 7,$ and 5 plants per morph per tissue type, respectively, except for seed fatty acids for which $n = 4$ per morph) were collected during 1999–2000, while flavonoid samples ($n = 5$ per morph per tissue type) were collected during 2001–2002. Extractions and analyses followed standard protocols; details may be found in Whitney (2003).

ANOVA and MANOVA (SAS Institute, 2000) were used to test the effect of morph colour on the chemical attributes of arils and seeds. Because cost considerations kept sample sizes low, population could not be included as a factor in the analysis. Ryan–Einot–Gabriel–Welsch (REGWQ) multiple range tests were used to assess the separation of means among morphs. For the elemental and lipid analyses, the large number of response variables (10 and 25, respectively) would result in low power if all were included in the analyses. For this reason, the number of dependent variables was reduced using principle components analysis (SAS Institute, 2000). Retention of principle components for use in the MANOVA was based on a scree plot of the eigenvalues (Sharma, 1996).

Power analyses

In ANOVA and MANOVA analyses where the data indicated that morphs did not differ, we were interested in the minimum effect sizes detectable with the sample sizes used. Retrospective power analyses were conducted with PASS 2002 software (Hintze, 2002). Power was set at ≥ 0.8 (corresponding to $\beta < 0.2$), type I error was set at 0.05, and population variances and covariances were estimated by the sample variances and covariances.

Results

Evidence for a genetic basis of the polymorphism

When grown in a common greenhouse environment, some maternal families were monomorphic for aril colour, while others contained two or three colour classes (Table 2). Moreover, pooled across maternal aril colours, aril colour of progeny was not independent of maternal aril colour (Fisher's exact test, $p = 0.005$). Over 90% of the progeny from red morphs were red, in contrast to 25 and 50% for orange and yellow morphs, respectively. Although cross-generation environmental effects could not be ruled out, this evidence is consistent with a genetic basis for the polymorphism.

Table 2. Frequencies of orange, red, and yellow-arilled plants in maternal families grown in a common greenhouse environment

Maternal aril colour	Family	No. plants		
		Orange	Red	Yellow
Orange	1	2	2	1
	2	3	0	0
Red	3	0	6	1
	4	0	1	0
	5	0	1	0
	6	0	2	0
	7	0	10	0
Yellow	8	0	1	2
	9	0	0	1
	10	0	1	0
	11	0	4	3

Paternal aril colours were unknown.

Seedling emergence and survival

Seedling emergence did not vary among morphs at any of the censuses ($F_{2,64} = 0.65$, $p = 0.53$, all interactions with time nonsignificant at $p > 0.2$). Least-squares estimates of mean final emergence fractions were 75.6% (± 0.07), 86.7% (± 0.03), and 86.7% (± 0.03) for orange, red, and yellow families, respectively (Fig. 2). Seeds derived from different populations exhibited different seedling emergence rates ($F_{2,64} = 7.58$, $p = 0.001$), but there was no interaction between population and morph ($F_{4,64} = 0.90$, $p = 0.47$). The analysis was sufficiently powerful to detect 22% differences in emergence fractions between red and yellow morphs, and 32% differences between orange and either red or yellow morphs.

Mortality of seedlings over the 14-month experiment was 98.6%, and the median survival time was 62 days. As expected from many other species (McGinley *et al.*, 1987), seed weight was positively associated with survival ($\chi^2 = 5.03$, $p = 0.025$) and so was retained as a covariate in the final model. However, maternal aril colour, population, and the interaction between them all failed to predict survival (Fig. 3; $\chi^2 = 0.74$, 1.66, and 3.88, respectively; $p = 0.69$, 0.44, and 0.42, respectively).

Clinal variation in morph frequencies

Red was the most frequent morph in all populations sampled (mean across populations = 71% of adult plants), followed by yellow (27%) and orange

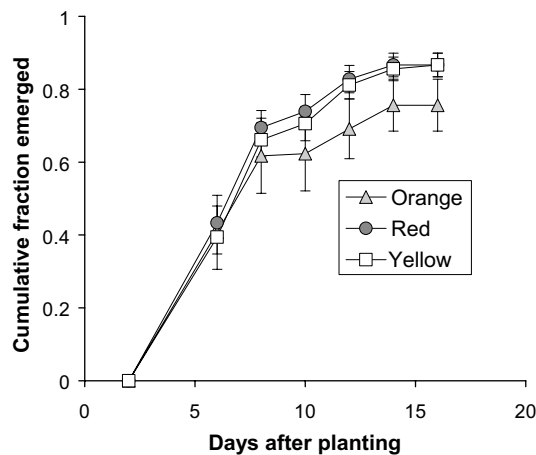


Figure 2. Emergence rates of seedlings by maternal aril colour, Cawndilla experimental plot. Least-squares means (\pm SE) across maternal families are shown. $n = 13$, 30, and 30 families for orange, red, and yellow morphs, respectively.

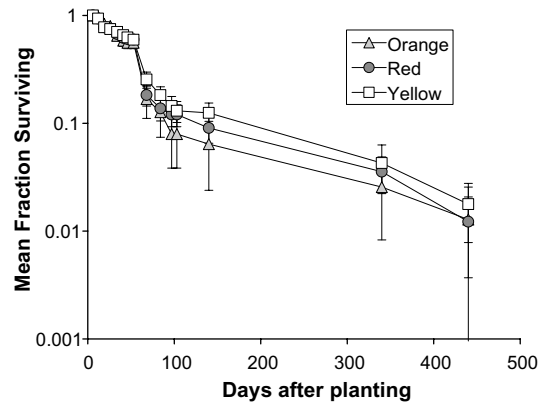


Figure 3. Seedling survival to 14 months by maternal aril colour, Cawndilla experimental plot. Means (\pm SE) across maternal families are shown. $n = 13, 30,$ and 30 families for orange, red, and yellow morphs, respectively.

(1%) (Table 1). Frequencies of the yellow morph decreased, as frequencies of the red morph increased, from north to south on the transect (Fig. 4). Latitude explained significant variation in morph frequencies (Pillai's Trace 0.59, $F_{2,8} = 5.65$, $p = 0.03$); this resulted from a close relationship between latitude and the frequency of both the yellow and red morphs (ANOVA, $F = 8.18$, $p = 0.02$). Latitude did not explain variation in frequencies of the rare orange morph (ANOVA, $F = 0.73$, $p = 0.41$). Neither landform (Pillai's Trace 0.16, $F_{2,8} = 0.75$, $p = 0.50$) nor the interaction between latitude and landform

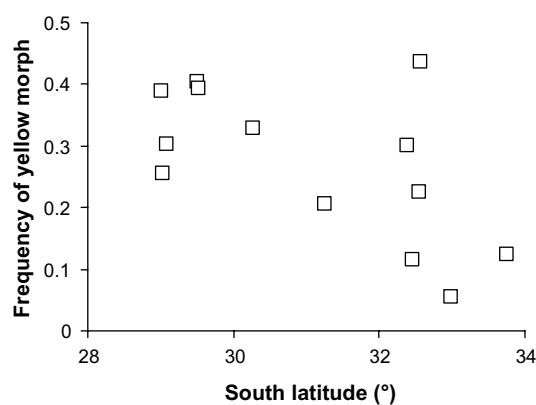


Figure 4. Latitudinal cline in the frequency of the yellow morph, western New South Wales ($F = 8.18$, $p = 0.02$). Frequencies of the red morph show the opposite pattern as they are tightly (and negatively) correlated with those of the yellow morph (see Methods).

(Pillai's Trace 0.18, $F_{2,8} = 0.86$, $p = 0.46$) were associated with variation in morph frequencies.

Consistent climatic changes occurred along the transect. As latitude increased in a southerly direction, mean annual rainfall increased (Pearson's $r = 0.969$, $p < 0.0001$) and temperature decreased ($r = -0.935$, $p = 0.02$). As longitude increased in an easterly direction, the similar gradients were observed (rainfall: $r = 0.939$, $p < 0.0001$; temperature: $r = -0.910$, $p = 0.03$). The tight intercorrelations between these climatic variables prevent us from distinguishing their individual relationships to clinal changes in morph frequencies.

Diaspore morphology

Neither seed mass nor aril mass varied significantly among morphs (Fig. 5; Pillai's Trace 0.66, $F_{4,8} = 0.98$, $p = 0.47$). Seed mass and aril mass did not differ among populations (Pillai's Trace 0.97, $F_{4,8} = 1.89$, $p = 0.21$), nor was there an interaction between population and morph (Pillai's Trace 0.14, $F_{8,128} = 1.21$, $p = 0.30$). The analysis was sufficiently powerful to detect 15% differences between red and yellow morph means, and 23% differences between means for orange and either red or yellow morphs.

Elemental, fatty acid and pigment chemistry of diaspores

Acacia ligulata colour morphs differed in nutritional chemistry (Table 3). Analysis of 10 macro- and microelements in aril tissue found significant variation among morphs (Pillai's Trace 0.6467, $F_{6,34} = 2.71$, $p = 0.03$), as deter-

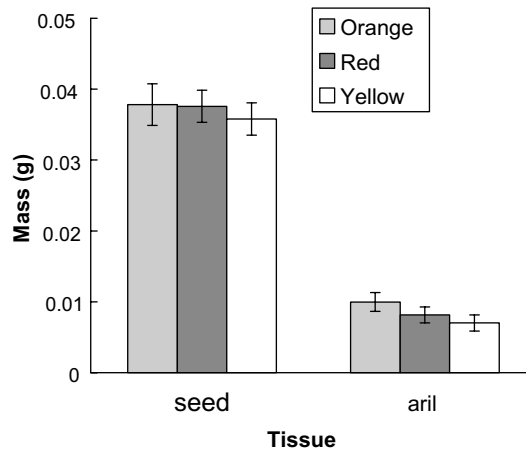


Figure 5. Seed and aril mass by maternal aril colour. Least-squares means (\pm SE) across maternal families are shown.

Table 3. Elemental composition of *A. ligulata* aril and seed tissue ($n = 7$ plants per morph)

Tissue/element	Orange morph	Red morph	Yellow morph
<i>Arils</i>			
Macroelements (%)			
N	3.91 ± 0.135	3.70 ± 0.193	3.61 ± 0.229
P	0.24 ^a ± 0.029	0.14 ^b ± 0.017	0.17 ^b ± 0.016
K	0.45 ± 0.084	0.44 ± 0.053	0.39 ± 0.040
Ca	0.03 ± 0.003	0.02 ± 0.003	0.03 ± 0.002
Mg	0.03 ^a ± 0.003	0.02 ^{a,b} ± 0.003	0.01 ^b ± 0.002
Microelements (ppm)			
B	16.86 ± 1.204	17.43 ± 1.429	16.14 ± 0.595
Zn	8.14 ± 0.769	16.71 ± 5.339	10.86 ± 2.773
Mn	3.87 ± 0.426	3.04 ± 0.485	2.54 ± 0.166
Fe	28.86 ± 5.726	42.43 ± 14.120	25.71 ± 4.586
Cu	2.57 ± 0.409	2.93 ± 0.354	3.03 ± 0.682
<i>Seeds</i>			
Macroelements (%)			
N	3.62 ± 0.114	3.65 ± 0.112	3.61 ± 0.046
P	0.35 ± 0.010	0.37 ± 0.012	0.39 ± 0.014
K	0.98 ± 0.044	0.99 ± 0.038	0.98 ± 0.024

Means ± SE are presented. Different letters indicate significant differences between morphs.

mined by MANOVA on three principle components encompassing 54.9% of the variation in the dependent variables. Morphs differed in aril concentrations of P ($F_{2,18} = 5.96$, $p = 0.01$) and Mg ($F_{2,18} = 5.07$, $p = 0.02$), and there was a trend for differences in Mn ($F_{2,18} = 3.04$, $p = 0.07$). Multiple range tests indicated significant separation between the orange morph and the other morphs. Orange had 40% higher concentrations of P and 200% higher concentrations of Mg than the yellow morph; additionally, it had 70% higher concentrations of P than the red morph (Table 3). There was also a trend in the orange morph for higher concentrations of Mn than the yellow morph (Table 3). No differences in aril tissue were found for the remaining elements ($p > 0.2$ in all cases). Seed tissue was analyzed for N, P, and K, and morphs did not differ (Table 3; Pillai's Trace 0.2991, $F_{6,32} = 0.94$, $p = 0.48$). The analysis was sufficiently powerful to detect a 31% difference among morph means for the elemental composition of seed tissue.

Morphs differed significantly in carotenoid chemistry ($F_{2,12} = 6.55$, $p = 0.01$). On average the red morph had 4.5 times the total carotenoids found in the yellow morph, while the orange morph had intermediate levels (Fig. 6). Multiple range tests indicated that red differed significantly in total carotenoids from orange and yellow, which did not differ from each other (Fig. 6). Individual compounds were not identified with the exception of β -carotene, which was more than twice as abundant in the red and orange morphs compared with the yellow morph (0.05 ± 0.010 , 0.06 ± 0.013 and 0.02 ± 0.006 mg/g,

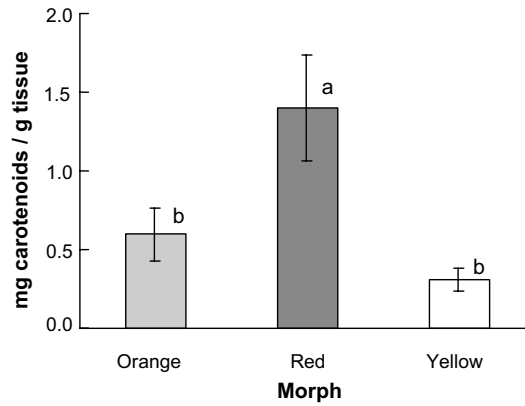


Figure 6. Total aril carotenoids by aril colour. Means (\pm SE) for $n = 5$ plants per morph are shown. Different letters indicate significant differences between morphs.

respectively; $F_{2,12} = 5.11$, $p = 0.02$). In addition, morphs differed greatly in a lycopene-like compound with a retention time of approximately 30.9 min. Average concentrations of this compound were over six times higher in the red morph than in either the orange or yellow morphs. Carotenoids were also examined in a limited number of samples of embryo ($n = 5$) and seed coat ($n = 3$) tissue. Embryos contained only 1.1% of the carotenoid concentrations found in arils, while seed coats contained no appreciable levels of carotenoids.

We found little variation among morphs in fatty acid composition (Appendix 1). Concentrations of 25 fatty acids were surveyed in both arils and seeds, and five principle components (encompassing 64.9% of the variation)

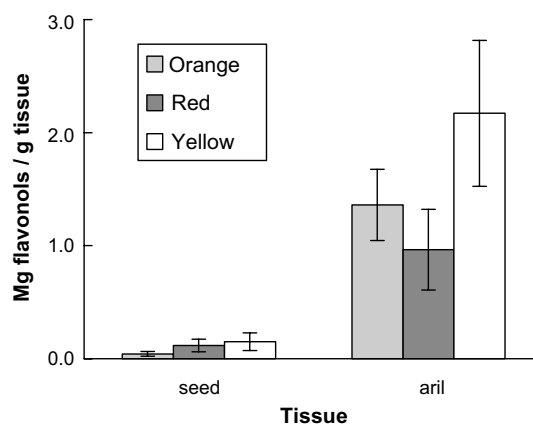


Figure 7. Total aril and seed flavonols by aril colour. Means (\pm SE) for $n = 5$ plants per morph are shown.

were analyzed in the MANOVA. While arils and seeds differed significantly in their fatty acid composition (Pillai's Trace 0.9536, $F_{5,23} = 94.46$, $p < 0.0001$), morphs did not differ (main effect: Pillai's Trace 0.3981, $F_{10,48} = 1.19$, $p = 0.32$; morph \times tissue type interaction: Pillai's Trace 0.2413, $F_{10,48} = 0.66$, $p = 0.76$). Because of high variance among plants, lipid principle component scores would have to differ by $\geq 65\%$ across morphs to have a high probability ($\beta < 0.2$) of producing a significant MANOVA result, given our sample sizes.

Flavonoids in both arils and seeds were predominantly quercetin glycosides and phenolic acids; no anthocyanins were detected, even in red morphs. Arils and seeds differed significantly in total flavonols (Fig. 7; $F_{1,24} = 74.46$, $p < 0.0001$). Although there was a trend for yellow morphs to have higher levels of total flavonols (Fig. 7), morphs did not differ significantly (main effect of morph: $F_{2,24} = 2.73$, $p = 0.09$; morph \times tissue type interaction: $F_{2,24} = 2.01$, $p = 0.16$). High sample-to-sample variability resulted in low power; $\geq 135\%$ differences among morph means would be required for a high probability of detection ($\beta < 0.2$) in this analysis.

Discussion

Genetics of the polymorphism

Examination of phenotypes of maternal half-sibships grown in a common environment indicated that aril colour in *A. ligulata* is likely under biparental genetic control, although the number of loci and alleles controlling the trait are currently unknown. Fruit colour in many plants is simply inherited (1–3 loci, Willson *et al.*, 1989). Furthermore, work with legumes has shown that maternal traits such as seed coat colour and pattern are frequently determined by a single locus (e.g., Mendel, 1865; Harding and Barnes, 1977). Further work is ongoing to more precisely determine the mode of inheritance of aril colour in *A. ligulata*.

Seedling emergence and survival

In the search for selective forces acting on fruit colour polymorphisms, studies have suggested that putative pleiotropic effects of colour alleles on seed germination rates may be important (Willson and O'Dowd, 1989; Gervais *et al.*, 1998; Traveset and Willson, 1998). Thus we investigated potential sources of selection on the *A. ligulata* polymorphism during early life history stages. Despite high mortality, however, we found no evidence of current selection. Rates of seedling emergence did not differ between progeny of *A. ligulata*

morphs under field conditions. Seedling survival in the field also did not differ between progeny of *A. ligulata* morphs. These results contrast with studies of the polymorphic species *Rhagodia parabolica* (Willson and O'Dowd, 1989) and *Rubus spectabilis* (Gervais *et al.*, 1998; Traveset and Willson, 1998), in which germination rates in the field varied among fruit colour morphs. These patterns could result from differences in fruit morphology. In *Rhagodia* and *Rubus*, seeds are enclosed within the pigmented tissue (in berries and drupelets, respectively), and thus may experience morph-specific chemical environments that impact seed viability or germination behaviour. In contrast, *A. ligulata* seeds are in contact with pigmented tissue only at their apices.

Clinal variation

Acacia ligulata represents the first known example of clinal variation in a fruit colour polymorphism. The yellow morph decreased in frequency along a gradient of decreasing maximum temperatures and increasing rainfall, while the red morph increased in frequency. While clinal patterns cannot provide conclusive evidence of selection (Hedrick, 1986), their existence is suggestive that the trait in question is not neutral (Mayr, 1965). The patterns suggest that climatic factors could have a large direct impact on relative fitness of colour morphs in this system. Alternatively, clinal variation could reflect gradients in biotic selective agents, as described below. A search for clines in other fruit-colour polymorphic species may be useful in identifying potential agents of selection on fruit colour traits.

Chemical variation suggests other potential sources of selection

While arils exhibited broad similarity across *A. ligulata* colour morphs in mass, lipids, and flavonoids, we found substantial differentiation of morphs in aril elements and carotenoids. Aril tissue of the orange morph contained higher concentrations of P and Mg than that of the yellow morph, and higher concentrations of P than the red morph. More strikingly, there were large quantitative and qualitative differences between morphs in carotenoid profiles. The concentration of total carotenoids was over four times higher in arils of the red morph relative to orange and yellow; in addition, each morph had characteristic concentrations of β -carotene and a lycopene-like compound. It has long been recognized that mutations can lead to carotenoid polymorphism in cultivated fruits (Goodwin and Goad, 1970). For example, alternate alleles at two loci in *Capsicum* affect the structure of synthases in the biosynthesis pathway of carotenoids, resulting in yellow, orange, and red fruits (Lefebvre *et al.*, 1998; Huh *et al.*, 2001). In contrast to the results for arils, we found that seeds of *A. ligulata* colour morphs were quite similar across an array of physical and

chemical characters. These characters included seed mass, elemental composition, lipids, flavonoids, and carotenoids.

Variation among *A. ligulata* morphs in elemental composition suggests that consumer preferences could generate selection on this and other fruit colour polymorphisms. While elemental differences between fruit colour morphs have yet to be found in other species (Willson and O'Dowd, 1989; Traveset and Willson, 1998; Gervais *et al.*, 1999; Traveset *et al.*, 2001), these earlier studies were characterized by small sample sizes, and may simply have lacked the power to detect differences among morphs. Slight differences in nutrient composition can have major effects on fruit preferences and assimilation efficiencies in frugivorous birds, especially if micronutrients are scarce (Levey and Martinez del Rio, 2001). The elements that differed between *A. ligulata* morphs – P, Mg, and possibly Mn – are all essential nutrients for birds (Murphy, 1996). We note, however, that the observed variation in elemental composition distinguished the orange morph from the red and yellow morphs, which did not differ from each other. As the orange morph is quite rare, the consequences of elemental variation for the dynamics of the polymorphism are not clear. Potentially, the orange morph's high investment in P and Mg may be metabolically costly, ultimately contributing to the relative scarcity of this morph if other factors (e.g., consumer preferences) do not increase its fitness relative to the other morphs.

Variation in pigment chemistry could also lead to selection on fruit colour polymorphisms. Willson and O'Dowd (1989) documented pigment differences among *Rhagodia parabolica* morphs, and hypothesized that those differences could affect both rates of fruit removal and plant defense. In *A. ligulata*, we found significant differences in carotenoid composition and levels between morphs. Because of their inability to synthesize carotenoids *de novo*, animals must typically obtain carotenoids from their diet (Kayser, 1985; Britton *et al.*, 1995). Certain carotenoids are precursors for vitamin A, which is necessary for proper functioning of vertebrate visual systems and plays a role in insect visual systems as well (Feltwell, 1978; Kayser, 1985). These provitamins include β -carotene, which was found in significantly higher concentrations in the red and orange morphs than in the yellow morph. Carotenoids are important in body colouration of both birds and insects, functioning in aposematic and sexual signaling (Feltwell, 1978; Rothschild, 1980; Olson and Owens, 1998; Hill, 2002). Carotenoids can play a variety of additional roles in insects; e.g. in defensive secretions (Feltwell, 1978), as agents protecting aposematic insects against toxic effects of sequestered chemicals (Rothschild *et al.*, 1986; Nishida *et al.*, 1994), and as antioxidants reducing the damaging effects of both UV radiation and phototoxic compounds from plant hosts (Kayser, 1985; Carroll *et al.*, 1997). Finally, biosynthetic links between pigments and defensive compounds suggest that the genetic differences underlying colour phenotypes could

also affect resistance to herbivores via pleiotropy (Willson and Whelan, 1990; Simms and Bucher, 1996).

Thus, chemical variation among fruit colour morphs (exemplified by that in *A. ligulata*) is variation that might matter to animal consumers, and suggests the possibility of selection by such diverse groups as seed dispersers, seed predators, and herbivores. If dispersal is advantageous, feeding bias in seed-dispersing species would presumably increase relative fitness of the preferred morph; in contrast, bias in seed predators and herbivores should act to decrease relative fitness of the preferred morph. Variation in the abundances or identities of these animals across environmental gradients could affect the relative fitnesses of colour morphs, potentially producing clinal variation in morph frequencies. Interestingly, Willson *et al.*, (1990) have documented shifts in the frequencies of plant species with various modes of dispersal (e.g., ant, vertebrate, wind) across environmental gradients. We suggest that a search for selective pressures imposed by seed dispersers, seed predators, and herbivores might shed light on the evolutionary dynamics of fruit colour polymorphisms; such studies are underway in the *A. ligulata* system.

Acknowledgements

For support and help of many kinds, many thanks to T. Auld, R. Taylor, J. Bean, A. Denham, and M. Ooi of the NSW National Parks and Wildlife Service. For dedicated assistance in the field we are grateful to I. Baird, E. Bjerre, J. Chranowski, M. Cooper, C. Donnelly, S. Enning, B. Fisher, C. Griffin, L. Grosche, B. Henshaw, K. Herman, M. Judd, S. Lee, E. Lindsay, A. McDonald, R. McIntyre, C. Menke, J. Nolte, P. Rymer, N. Wurzburger, K. Yee and M. Zeppel. Thanks to B. German, B. Burri, T. Neidlinger, K. Sutton and E. Kim for chemical analyses, and to D. Walker, T. Metcalf, E. Sandoval, M. Nguyen for greenhouse support. N. Willits provided statistical advice. Thanks to J. Rudgers for advice and loads of help. E. Baack, J. Rudgers, T. Smith, M. Stanton and S. Strauss provided helpful comments on the manuscript. This work was supported by the Center for Population Biology (UC Davis) and an EPA STAR Fellowship to K.D.W.

Appendix 1

Fatty acid composition (mg/g) of *A. ligulata* aril and seed tissue

Tissue/fatty acid	Orange morph	Red morph	Yellow morph
<i>Arils</i>			
Myristic	1.15 ± 0.246	0.84 ± 0.199	1.15 ± 0.214
Myristoleic	0.03 ± 0.014	0.00 ± 0.000	0.00 ± 0.000

Appendix 1. (Continued)

Tissue/fatty acid	Orange morph	Red morph	Yellow morph
Palmitic	87.22 ± 16.801	128.44 ± 26.016	118.74 ± 7.870
Palmitoleic	10.04 ± 2.784	10.79 ± 2.462	10.62 ± 4.689
Stearic	9.78 ± 1.180	11.06 ± 1.590	7.32 ± 2.111
Oleic	220.68 ± 9.931	231.67 ± 16.328	234.99 ± 18.250
Vaccenic	0.00 ± 0.000	0.00 ± 0.000	8.92 ± 8.922
Linolenic	11.26 ± 1.240	14.52 ± 2.458	12.97 ± 1.500
Gamma-linolenic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Alpha-linolenic	3.62 ± 0.536	3.01 ± 0.320	3.83 ± 0.349
Arachidic	1.65 ± 0.173	1.42 ± 0.322	1.40 ± 0.349
Eicosenoic	0.43 ± 0.080	0.26 ± 0.131	0.39 ± 0.160
Eicosadienoic	0.05 ± 0.037	0.00 ± 0.000	0.06 ± 0.065
Homo-gamma-linolenic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Arachidonic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Eicosatrienoic	0.11 ± 0.107	0.00 ± 0.000	0.00 ± 0.000
Eicosapentaenoic	0.03 ± 0.029	0.00 ± 0.000	0.00 ± 0.000
Behenic	0.54 ± 0.070	0.35 ± 0.207	0.24 ± 0.155
Erucic	0.05 ± 0.024	0.00 ± 0.000	0.00 ± 0.000
Docosadienoic	0.02 ± 0.022	0.04 ± 0.044	0.00 ± 0.000
Docosatetraenoic	0.01 ± 0.008	0.00 ± 0.000	0.00 ± 0.000
Docosapentaenoic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Docosahexaenoic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Lignoceric	1.69 ± 0.685	0.29 ± 0.188	0.05 ± 0.054
Nervoic	0.36 ± 0.363	0.30 ± 0.296	0.00 ± 0.000
<i>Seeds</i>			
Myristic	0.05 ± 0.003	0.06 ± 0.004	0.05 ± 0.005
Myristoleic	0.01 ± 0.011	0.01 ± 0.011	0.00 ± 0.000
Palmitic	6.17 ± 0.357	7.18 ± 0.084	7.27 ± 0.352
Palmitoleic	0.13 ± 0.018	0.16 ± 0.013	0.12 ± 0.008
Stearic	2.06 ± 0.260	2.13 ± 0.374	3.16 ± 0.624
Oleic	15.78 ± 0.669	17.10 ± 2.200	18.66 ± 1.662
Vaccenic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Linolenic	63.72 ± 1.839	71.03 ± 4.770	66.16 ± 3.731
Gamma-linolenic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Alpha-linolenic	0.29 ± 0.014	0.35 ± 0.046	0.38 ± 0.029
Arachidic	1.21 ± 0.086	1.42 ± 0.089	1.55 ± 0.173
Eicosenoic	0.05 ± 0.053	0.00 ± 0.000	0.00 ± 0.000
Eicosadienoic	0.02 ± 0.022	0.00 ± 0.000	0.00 ± 0.000
Homo-gamma-linolenic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Arachidonic	0.00 ± 0.000	0.00 ± 0.000	0.12 ± 0.075
Eicosatrienoic	0.02 ± 0.016	0.00 ± 0.000	0.04 ± 0.027
Eicosapentaenoic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Behenic	0.75 ± 0.260	1.11 ± 0.070	1.03 ± 0.073
Erucic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Docosadienoic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Docosatetraenoic	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Docosapentaenoic	0.70 ± 0.702	0.00 ± 0.000	0.00 ± 0.000
Docosahexaenoic	0.01 ± 0.011	0.02 ± 0.018	0.00 ± 0.000
Lignoceric	0.50 ± 0.178	0.36 ± 0.232	0.46 ± 0.155
Nervoic	0.01 ± 0.006	0.00 ± 0.000	0.00 ± 0.000

Means ± SE are presented. $n = 7$ plants per morph (aril tissue) and 4 plants per morph (seed tissue).

References

- Auld, T.D. (1995a) Soil seedbank patterns of four trees and shrubs from arid Australia. *J. Arid Env.* **29**, 33–45.
- Auld, T.D. (1995b) The impact of herbivores on regeneration in four trees from arid Australia. *Rangeland J.* **17**, 213–227.
- Auld, T.D. and Denham, A.J. (2001) Flora conservation issues at Kinchega National Park, western NSW. *Cunninghamia* **7**, 27–41.
- Britton, G., Liaaen-Jensen, S. and Pfander, H. (1995) Carotenoids today and challenges for the future. In G. Britton, S. Liaaen-Jensen and H. Pfander (eds) *Carotenoids*. Vol. 1A. Birkhauser Verlag, Basel, pp. 13–26.
- Carroll, M., Hanlon, A., Hanlon, T., Zangerl, A.R. and Berenbaum, M.R. (1997) Behavioral effects of carotenoid sequestration by the parsnip webworm, *Depressaria pastinacella*. *J. Chem. Ecol.* **23**, 2707–2719.
- Chapman, A.R. and Maslin, B.R. (1992) *Acacia* miscellany 5: a review of the *A. bivenosa* group (Leguminosae: Mimosoideae: Section *Phyllodineae*). *Nuytsia* **8**, 249–283.
- Davidson, D.W. and Morton, S.R. (1984) Dispersal adaptations of some *Acacia* species in the Australian arid zone. *Ecology* **65**, 1038–1051.
- Epling, C. and Dobzhansky, T. (1942) Genetics of natural populations: VI. Microgeographical races in *Linanthus parryae*. *Genetics* **27**, 317–332.
- Feltwell, J. (1978) The distribution of carotenoids in insects. In J.B. Harborne (ed.) *Biochemical Aspects of Plant and Animal Coevolution*. Academic Press, London, pp. 277–293.
- Forde, N. (1986) Relationships between birds and fruits in temperate Australia. In H.A. Ford and D.C. Paton (eds) *The Dynamic Partnership: Birds and Plants in Southern Australia*. Government Printer, South Australia, pp. 42–58.
- Gervais, J.A., Noon, R.B. and Willson, M.F. (1999) Avian selection of the color-dimorphic fruits of salmonberry, *Rubus spectabilis*: a field experiment. *Oikos* **84**, 77–86.
- Gervais, J.A., Traveset, A. and Willson, M.F. (1998) The potential for seed dispersal by the banana slug (*Ariolimax columbianus*). *Am. Midl. Nat.* **140**, 103–110.
- Gillespie, J.H. (1998) *Population Genetics: A Concise Guide*. Johns Hopkins University Press, Baltimore.
- Goodwin, T.W. and Goad, L.J. (1970) Carotenoids and triterpenoids. In A.C. Hulme (ed.) *The Biochemistry of Fruits and Their Products*. Vol. 1. Academic Press, London, pp. 305–368.
- Groos, C., Gay, G., Perretant, M.-R., Gervais, L., Bernard, M., Dedryver, F. and Charmet, G. (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. *Theor. Appl. Genet.* **104**, 39–47.
- Harding, J. and Barnes, K. (1977) Genetics of *Lupinus*. X. Genetic variability, heterozygosity and outcrossing in colonial populations of *Lupinus succulentus*. *Evolution* **31**, 247–255.
- Hedrick, P.W. (1986) Genetic polymorphism in heterogeneous environments: a decade later. *Ann. Rev. Ecol. Syst.* **17**, 535–566.
- Hill, G.E. (2002) *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. Oxford University Press, New York.
- Hintze, J. (2002) *PASS*. Number Cruncher Statistical Systems, Kaysville, Utah, USA.
- Huh, J.H., Kang, B.C., Nahm, S.H., Kim, S., Ha, K.S., Lee, M.H. and Kim, B.D. (2001) A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.). *Theor. Appl. Genet.* **102**, 524–530.
- Kayser, H. (1985) Pigments. In G.A. Kerkut and L.E. Gilbert (eds) *Comparative Insect Physiology, Biochemistry, and Pharmacology*. Vol. 10. Academic Press, New York, pp. 368–415.
- Lefebvre, V., Kuntz, M., Camara, B. and Palloix, A. (1998) The capsanthin-capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Mol. Biol.* **36**, 785–789.
- Letnic, M., Dickman, C.R. and McNaught, G. (2000) Bet-hedging and germination in the Australian arid zone shrub *Acacia ligulata*. *Aust. Ecol.* **25**, 368–374.

- Levey, D.J. and Martinez del Rio, C. (2001) It takes guts (and more) to eat fruit: lessons from avian nutritional ecology. *Auk* **118**, 819–831.
- Maslin, B.R. and Hopper, S.D. (1982) Phytogeography of *Acacia* (Leguminosae: Mimosoideae) in Central Australia. In W.R. Barker and P.J.M. Greenslade (eds) *Evolution of the Flora and Fauna of Arid Australia*. Peacock Publications, Frewville, South Australia, pp. 301–315.
- Mayr, E. (1965) *Animal Species and Evolution*. Belknap Press, Cambridge, MA, USA.
- McGinley, M.A., Temme, D.H. and Geber, M.A. (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *Am. Nat.* **130**, 370–398.
- Mendel, G. (1865) *Experiments in Plant-hybridisation*. Harvard University Press Edition, 1941, Cambridge.
- Murphy, M.E. (1996) Nutrition and metabolism. In C. Carey (ed.) *Avian Energetics and Nutritional Ecology*. Chapman and Hall, New York, pp. 31–60.
- Nishida, R., Rothschild, M. and Mummery, R. (1994) A cyanoglucoside, sarmentosin, from the Magpie Moth, *Abraxas grossulariata*, Geometridae: Lepidoptera. *Phytochemistry* **36**, 37–38.
- O'Dowd, D.J. and Gill, A.M. (1986) Seed dispersal syndromes in Australian *Acacia*. In D.R. Murray (ed.) *Seed Dispersal*. Academic Press, San Diego, California, pp. 87–122.
- Olson, V.A. and Owens, I.P.F. (1998) Costly sexual signals: are carotenoids rare, risky, or required? *Trends Ecol. Evol.* **13**, 510–514.
- Philippi, T.E. (1993) Multiple regression: herbivory. In S.M. Scheiner and J. Gurevitch, (eds) *Design and Analysis of Ecological Experiments*. Chapman and Hall, New York, NY, pp. 183–210.
- Puckey, H.L., Lill, A. and O'Dowd, D.J. (1996) Fruit color choices of captive silvereyes (*Zosterops lateralis*). *Condor* **98**, 780–790.
- Rothschild, M. (1980) Remarks on carotenoids in the evolution of signals. In L.E. Gilbert and P.H. Raven, (eds) *Coevolution of Animals and Plants*. Revised Edition, University of Texas Press, Austin, pp. 20–50.
- Rothschild, M., Mummery, R. and Farrell, C. (1986) Carotenoids of butterfly models and their mimics (Lep: Papilionidae and Nymphalidae). *Biol. J. Linn. Soc.* **28**, 359–372.
- SAS Institute (2000) *The SAS System for Windows*, release 8.1. SAS Institute, Cary, NC, USA.
- Scheiner, S.M. (1993) MANOVA: multiple response variables and multispecies interactions. In S.M. Scheiner and J. Gurevitch, (eds) *Design and Analysis of Ecological Experiments*. Oxford University Press, Oxford, pp. 94–112.
- Sharma, S. (1996) *Applied Multivariate Techniques*. Wiley, New York.
- Sheppard, P.M. (1951) Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepea nemoralis*. *Heredity* **5**, 125–134.
- Simms, E.L. and Bucher, M.A. (1996) Pleiotropic effects of flower-color intensity on herbivore performance on *Ipomoea purpurea*. *Evolution* **50**, 957–963.
- Traveset, A., Riera, N. and Mas, R.E. (2001) Ecology of fruit-color polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. *J. Ecol.* **89**, 749–760.
- Traveset, A. and Willson, M.F. (1998) Ecology of the fruit-colour polymorphism in *Rubus spectabilis*. *Evol. Ecol.* **12**, 331–345.
- Whitney, K.D. (2002) Dispersal for distance? *Acacia ligulata* seeds and meat ants *Iridomyrmex viridiaeneus*. *Austral Ecol.* **27**, 589–595.
- Whitney, K.D. (2003) Evolutionary ecology of seed predation and seed dispersal in a polymorphic acacia. *Ph.D. Dissertation*, University of California, Davis, California.
- Willson, M.F. (1986) Avian frugivory and seed dispersal in eastern North America. *Curr. Ornithol.* **3**, 223–279.
- Willson, M.F. (1994) Fruit choices by captive American robins. *Condor* **96**, 494–502.
- Willson, M.F. and Comet, T.A. (1993) Food choices by northwestern crows: experiments with captive, free-ranging and hand-raised birds. *Condor* **95**, 596–615.
- Willson, M.F. and O'Dowd, D.J. (1989) Fruit color polymorphism in a bird-dispersed shrub (*Rhagodia parabolica*) in Australia. *Evol. Ecol.* **3**, 40–50.
- Willson, M.F. and Whelan, C.J. (1990) The evolution of fruit color in fleshy-fruited plants. *Am. Nat.* **136**, 790–809.

- Willson, M.F., Irvine, A.K. and Walsh, N.G. (1989) Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographic comparisons. *Biotropica* **21**, 133–147.
- Willson, M.F., Rice, B.L. and Westoby, M. (1990) Seed dispersal spectra: a comparison of temperate plant communities. *J. Veg. Sci.* **1**, 547–562.