

Short Communication

Evidence for simple genetic control of a fruit-colour polymorphism in *Acacia ligulata*

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Abstract. Fruit-colour polymorphisms are common in nature, but their genetic bases have rarely been examined in wild species. Here, I report on controlled crosses in *Acacia ligulata* A.Cunn. ex Benth., an Australian arid-zone shrub with a red–yellow–orange aril colour polymorphism. The evidence is consistent with 1-locus, 2-allele control of red *v.* yellow phenotypes; these phenotypes comprise 98.7% of the adult plants in nature. At this proposed *r* locus, yellow is dominant to red. Evidence concerning the rare orange morph is limited, but is consistent with models in which orange is produced by either (a) a third allele at the *r* locus or (b) modification by a second locus. Simple genetic architecture for ecologically relevant traits, such as fruit colour, should aid in linking ecological processes such as frugivory and seed dispersal to the evolutionary trajectories of plant populations.

Introduction

Fruit-colour polymorphisms, in which mature fruit colour differs discretely among members of a population, occur on most continents and in at least 19 different plant families (Forde 1986; Willson 1986; Willson *et al.* 1989). Such polymorphisms are of interest because they provide a window to the maintenance of genetic diversity in nature. Recent studies of fruit-colour polymorphisms have shown that, in field settings, animal consumers can interact differentially with morphs (e.g. frugivores: Willson and O’Dowd 1989; Gervais *et al.* 1998, 1999; Whitney 2005; seed predators: Whitney and Stanton 2004), and that morphs can respond differentially to environmental conditions (e.g. soil types; Traveset and Willson 1998). In order to understand how these biotic and abiotic factors might influence the ratio of colour morphs within populations and, ultimately, the persistence of polymorphisms, more information is needed on the genetic bases of colour phenotypes.

In crop species such as tomato and pepper, substantial work has documented the mutations underlying alternative pigment biosynthetic pathways and the resulting fruit-colour variation (e.g. Thorup *et al.* 2000 and references therein; Huh *et al.* 2001; Paris 2002; Borovsky *et al.* 2004; Lang *et al.* 2004). In contrast, very little is known about

the genetic architecture of fruit-colour variation in wild species. In North American *Vaccinium myrtilloides* and *V. angustifolium*, similar white–blue fruit-colour polymorphisms were found to be underlain by one and two loci, respectively (Aalders and Hall 1962; Hall and Aalders 1963).

In the current study, I examine the genetic basis of fruit-colour polymorphism in *Acacia ligulata*, a widespread Australian dune shrub with a red–yellow–orange aril colour polymorphism (Whitney and Lister 2004). Previous work has demonstrated that avian seed dispersers and predators, as well as insect seed predators, can discriminate between the colour morphs during feeding and alter relative fitness of the morphs (Whitney and Stanton 2004; Whitney 2005). The evidence is qualitatively consistent with long-term maintenance of the polymorphism via such variable selection; red and yellow morphs each have the highest seed production in alternative sites (Whitney and Stanton 2004).

Materials and methods

Acacia ligulata is a widespread dune shrub of arid Australia (Maslin and Hopper 1982), reaching up to 5 m in height. Flowers are yellow and are borne in globose inflorescences in the axils of the phyllodes. The index of self-incompatibility (ratio of seeds set after self-pollination to those set after cross-pollination) is 0.06 (K. D. Whitney, unpubl. data),

indicating a high degree of self-incompatibility, consistent with other members of the genus (Kenrick and Knox 1989). Also in common with other *Acacia* species (Stone *et al.* 2003), *A. ligulata* is visited by generalist pollinating insects such as flies and feral honeybees *Apis mellifera* (pers. obs.); the role of native bees is not known. Diaspores of *A. ligulata* are bicoloured: a black seed (c. 5 mm in length) contrasts with an expanded, coloured funicle (the aril or elaiosome, c. 2.5 mm). A given plant produces arils of single colour, either red, yellow or orange (for an image depicting the polymorphism, see the online appendix accompanying Whitney, in press). New South Wales populations are dominated by red morphs (71% of adults), followed by yellow (27%) and orange (1%) (Whitney and Lister 2004). Comparisons of the colour morphs have found no differences in mass or fatty acid composition of arils or seeds. However, arils of red morphs have higher concentrations of carotenoids than those of yellow or orange morphs; additionally, arils of orange morphs are distinguished by elevated levels of phosphorus and magnesium (Whitney and Lister 2004). At maturity, diaspores are displayed on the valves of the dehiscent pods, and if not removed, eventually fall to the ground. A variety of ants and birds are attracted to the lipid-rich arils and disperse the seeds (Whitney 2005, and references therein).

Crosses among *A. ligulata* plants growing naturally in the field were carried out during 3–28 September 1999 in Kinchega National Park, New South Wales, Australia. Crosses used individuals in two populations (Big Dune and Menindee; for site details, see Whitney and Lister 2004) for which aril-colour phenotypes were scored the previous year. Thirteen dams were chosen, comprising five red, five yellow and three orange morphs. Eight inflorescence-bearing branches per dam were chosen and bagged with polyester plant sleeves (Fibe-Air 19 × 48 × 58 cm, Kleen Test Products, Wisconsin, USA). Inflorescences that were open at the time of bagging were removed from the plant, after which each branch bore an average of 56 inflorescences. Each dam received pollen from two sires (three dam branches per sire) as well as self-pollen (two dam branches). Each day, pollen from 10–50 inflorescences per sire was collected by tapping inflorescences above a glass microscope slide. Pollen was pushed into a ridge at one end of the slide by using the edge of a clean insect pin, and was then applied to stigmas of bagged inflorescences on the dam. Resulting seeds were collected in December 1999.

Seeds were planted into a greenhouse in Davis, California, USA, in May 2000 after dormancy was broken with a 1-min boiling treatment. Plants were grown in modified 'UC Mix' (a University of California standard soil mixture) in 3.8-L pots and were watered with a solution of distilled water and fertiliser. Of 783 initial seeds, ~320 survived to the juvenile stage. A small fraction of these F₁ progeny flowered between July 2001 and March 2002. These were hand-pollinated with mixed pollen from randomly chosen donors and aril colour was assessed at fruit maturity. This resulted in known phenotypes for 20 F₁ progeny (6%). Given this low success rate in the greenhouse, I transplanted progeny to the field in the hope that larger plant sizes attained outside of pots would induce flowering and fruiting. Because space limitations required planting only a subset of the progeny, preference was given to crosses involving the numerically dominant morphs (red and yellow). During 7–10 April 2002, 142 F₁ plants were transplanted into an open field in Davis in an 11 × 13-m grid with 1-m spacing. Plants were irrigated for the first 5 months and were allowed to open-pollinate. Fruit set was minimal in 2002 and 2003 but was substantial in 2004, 4 years following the initial planting. Aril colour was scored in late August 2004, resulting in known aril-colour phenotypes for an additional 87 F₁ plants.

Deviations from Mendelian ratios were tested with Fisher's exact tests (according to the procedure Proc FREQ; SAS Institute 2000), which unlike traditional χ^2 tests are appropriate even when expected values are <5 (Stokes *et al.* 2000).

Results

Red and yellow morphs

Data on phenotypes of parental and F₁ plants are given in Table 1. Strikingly, the two crosses between red morphs produced almost exclusively red progeny (42 of 43 progeny). Assuming that the single yellow progeny is the result of a methodological error (e.g. pollen contamination, mislabelling), this suggested the preliminary hypothesis that the red phenotype is a double recessive (*rr*), whereas the yellow phenotype is produced by *Rr* and *RR* genotypes.

This hypothesis was further tested by examining data from yellow × yellow and red × yellow crosses for which sufficient numbers of phenotyped progeny were obtained. A yellow × yellow cross would be expected to produce either all yellow progeny (if one or both parents were *RR*) or yellow and red in the ratio 3 : 1 (if both parents were *Rr* heterozygotes). Consistent with this prediction, Cross 9 produced progeny in a 4.25 : 1 ratio, not significantly different from 3 : 1 (Fisher's exact test, $P = 0.624$). A red × yellow cross would be expected to produce either all yellow progeny (if the yellow parent were *RR*) or yellow and red in the ratio 1 : 1 (if the yellow parent were a *Rr* heterozygote). Consistent with the hypothesis, Cross 7 produced a 2.3 : 1 ratio, not significantly different from 1 : 1 (Fisher's exact test, $P = 0.115$). If the results from all three red × yellow crosses producing some red progeny (Crosses 7, 19 and 28) are pooled, the ratio is 1.2 : 1, again not significantly different from 1 : 1 (Fisher's exact test, $P = 0.711$).

The hypothesis was then applied to previously published data on aril colours of plants germinated from open-pollinated seeds collected in the field (Whitney and Lister

Table 1. Aril-colour phenotypes of progeny from *Acacia ligulata* crosses

'Cross type' lists the phenotypes of the dam and sire in order

Cross no.	Cross type	Dam ID	Sire ID	Number of progeny		
				Orange	Red	Yellow
11	O self	101	101		1	
20	O × O	173	101	3		1
12	O × R	101	113	1		
17	Y × O	162	173	1		
3	R self	33	33		1	
6	R × R	35	31		18	1
27	R × R	184	169		24	
8	Y self	36	36			1
2	Y × Y	30	36		1	1
9	Y × Y	36	21		4	17
23	Y × Y	175	185			1
24	Y × R	175	179			2
19	R × Y	167	175		2	
28	R × Y	184	176		5	2
7	R × Y	35	30		6	14

2004). Thirty of these seeds (all but Families 1, 2, 6 and 10 of Whitney and Lister 2004) were collected from a population of 69.8% red, 30.2% yellow and 0% orange morphs. Assuming Hardy–Weinberg equilibrium, the frequency of the *r* allele should be 0.84. Assuming random mating, we predict that red dams should produce progeny in a yellow to red ratio of 1 : 5.1. The actual ratio was 1 : 18 ($n = 19$ progeny) and did not differ significantly from predictions (Fisher's exact test, $P = 0.347$). In turn, yellow dams should produce a 1.6 : 1 ratio; the actual ratio of 1.2 : 1 ($n = 11$ progeny) also did not differ significantly from predictions (Fisher's exact test, $P = 0.758$).

Orange morphs

Although data pertaining to orange morphs are limited, some hypotheses can be proposed. The data clearly contradict the hypothesis that orange is a heterozygote at a diallelic *r* locus; none of the 31 progeny of red \times yellow crosses was orange. However, the data are consistent with two alternative models. The orange phenotype could be produced by a third allele at the *r* locus. Because orange \times orange crosses (Crosses 11, 20) produced both red and yellow progeny in addition to orange, the orange allele would have to be dominant to the remaining alleles, to wit *rr* red; R^1r , R^1R^1 yellow; and R^2r , R^1R^2 , R^2R^2 orange. Alternatively, the orange phenotype could result from a second locus acting epistatically on the first.

Discussion

The data support the hypothesis that the red–yellow–orange fruit-colour polymorphism in *A. ligulata* has a relatively simple genetic basis, with the yellow allele (*R*) dominant to the red allele (*r*). Although limited, the data are also consistent with the orange morph being produced either by a third allele at the *r* locus or by alleles at a second locus. These hypotheses require further testing (e.g. via F_1 crosses).

Acacia ligulata morphs are distinguished by large quantitative and qualitative differences in carotenoid, but not flavonoid, profiles (Whitney and Lister 2004). The concentration of total carotenoids is over four times higher in arils of the red morph than in the orange and yellow; in addition, each morph has characteristic concentrations of individual compounds such as β -carotene (high in red and orange, low in yellow morphs) and a lycopenelike compound (high in red, low in yellow and orange morphs). These patterns indicate that the putative *r* locus in *A. ligulata* probably acts on the structure or regulation of enzymes in the carotenoid biosynthesis pathway. As such, it may have similarities to carotenoid polymorphisms in cultivated Solanaceae (Thorup *et al.* 2000). In cultivated pepper (*Capsicum*), three loci (*y*, *c1*, *c2*) are hypothesised to control yellow, orange and red phenotypes of mature fruit (Hurtado-Hernandez and Smith 1985). These loci

have been linked to particular enzymes (Lefebvre *et al.* 1998; Huh *et al.* 2001; Lang *et al.* 2004). For example, the *y* locus has been linked to the capsanthin–capsorubin synthesis gene (CCS); in some accessions, red-fruited plants have normal CCS function and normal levels of the carotenoid capsanthin, whereas yellow- and orange-fruited plants have a deletion in this gene and are deficient in capsanthin (Lefebvre *et al.* 1998; Lang *et al.* 2004). Thus, there is the potential for candidate gene approaches being used to further investigate the proposed *r* locus in *A. ligulata*.

Tracing the effects of allelic substitutions on the ecology and evolution of organisms in nature is a widely held goal (Lowe *et al.* 2004). The *A. ligulata* system demonstrates that fruit-colour polymorphisms can be useful in pursuing this goal, as the genetic basis is apparently simple and the fitness consequences are measurable in the field (Whitney and Stanton 2004; Whitney 2005). Future efforts will combine information on the genetic basis of the trait with spatiotemporal patterns of selection to predict the long-term dynamics of the polymorphism.

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References

- Aalders LE, Hall IV (1962) The inheritance of white fruit in the velvet-leaf blueberry, *Vaccinium myrtilloides* Michx. *Canadian Journal of Genetics and Cytology* **4**, 90–91.
- Borovsky Y, Oren-Shamir M, Ovadia R, De Jong W, Paran I (2004) The *A* locus that controls anthocyanin accumulation in pepper encodes a MYB transcription factor homologous to anthocyanin2 of petunia. *Theoretical and Applied Genetics* **109**, 23–29. doi: 10.1007/s00122-004-1625-9
- Forde N (1986) Relationships between birds and fruits in temperate Australia. In 'The dynamic partnership: birds and plants in southern Australia'. (Eds HA Ford, DC Paton) pp. 42–58. (Government Printer: Adelaide)
- Gervais JA, Traveset A, Willson MF (1998) The potential for seed dispersal by the banana slug (*Ariolimax columbianus*). *American Midland Naturalist* **140**, 103–110.
- Gervais JA, Noon RB, Willson MF (1999) Avian selection of the color-dimorphic fruits of salmonberry, *Rubus spectabilis*: a field experiment. *Oikos* **84**, 77–86.
- Hall IV, Aalders LE (1963) Two-factor inheritance of white fruit in the common lowbush blueberry, *Vaccinium angustifolium* Ait. *Canadian Journal of Genetics and Cytology* **5**, 371–373.

- Huh JH, Kang BC, Nahm SH, Kim S, Ha KS, Lee MH, Kim BD (2001) A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.). *Theoretical and Applied Genetics* **102**, 524–530. doi: 10.1007/s001220051677
- Hurtado-Hernandez H, Smith PG (1985) Inheritance of mature fruit color in *Capsicum annum* L. *Journal of Heredity* **76**, 211–213.
- Kenrick J, Knox RB (1989) Quantitative analysis of self-incompatibility in trees of seven species of *Acacia*. *Journal of Heredity* **80**, 240–245.
- Lang Y, Yanagawa S, Sasanuma T, Sasakuma T (2004) Orange fruit color in *Capsicum* due to deletion of capsanthin-capsorubin synthesis gene. *Breeding Science* **54**, 33–39. doi: 10.1270/jsbbs.54.33
- Lefebvre V, Kuntz M, Camara B, Palloix A (1998) The capsanthin–capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Molecular Biology* **36**, 785–789. doi: 10.1023/A:1005966313415
- Lowe A, Harris S, Ashton P (2004) 'Ecological genetics: design, analysis, application.' (Blackwell Publishing: Oxford, UK)
- Maslin BR, Hopper SD (1982) Phylogeography of *Acacia* (Leguminosae: Mimosoideae) in Central Australia. In 'Evolution of the flora and fauna of arid Australia'. (Eds WR Barker, PJM Greenslade) pp. 301–315. (Peacock Publications: Frewville, SA)
- Paris HS (2002) Multiple allelism at a major locus affecting fruit coloration in *Cucurbita pepo*. *Euphytica* **125**, 149–153. doi: 10.1023/A:1015898305507
- SAS Institute (2000) 'The SAS system for Windows, release 8.1.' (SAS Institute: Cary, NC)
- Stokes ME, Davis CS, Koch GG (2000) 'Categorical data analysis using the SAS system.' (SAS Institute Inc.: Cary, NC)
- Stone GN, Raine NE, Prescott M, Willmer PG (2003) Pollination ecology of acacias (Fabaceae, Mimosoideae). *Australian Systematic Botany* **16**, 103–118. doi: 10.1071/SB02024
- Thorup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M (2000) Candidate gene analysis of organ pigmentation loci in the Solanaceae. *Proceedings of the National Academy of Sciences, USA* **97**, 11 192–11 197. doi: 10.1073/pnas.97.21.11192
- Traveset A, Willson MF (1998) Ecology of the fruit-colour polymorphism in *Rubus spectabilis*. *Evolutionary Ecology* **12**, 331–345. doi: 10.1023/A:1006504317585
- Whitney KD (2005) Linking frugivores to the dynamics of a fruit color polymorphism. *American Journal of Botany* **92**, 859–867.
- Whitney KD, Lister CE (2004) Fruit colour polymorphism in *Acacia ligulata*: seed and seedling performance, clinal patterns, and chemical variation. *Evolutionary Ecology* **18**, 165–186. doi: 10.1023/B:EVEC.0000021153.64497.c1
- Whitney KD, Stanton ML (2004) Insect seed predators as novel agents of selection on fruit color. *Ecology* **85**, 2153–2160.
- Willson MF (1986) Avian frugivory and seed dispersal in eastern North America. *Current Ornithology* **3**, 223–279.
- Willson MF, O'Dowd DJ (1989) Fruit color polymorphism in a bird-dispersed shrub (*Rhagodia parabolica*) in Australia. *Evolutionary Ecology* **3**, 40–50.
- Willson MF, Irvine AK, Walsh NG (1989) Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographic comparisons. *Biotropica* **21**, 133–147.

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