Ecology of the fruit-colour polymorphism in *Rubus spectabilis*

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Summary

Although some studies have focused on the colour polymorphisms of flowers and fruits, little is known of their ecological and evolutionary significance. We investigated the potential contribution of several factors to the maintenance of fruit-colour polymorphism in *Rubus spectabilis*, a common shrub in the temperate rainforests of southeast Alaska. Fruits occur in two colours (red and orange), whose frequencies vary geographically. The two colour morphs have similar size, weight, seed load and nutrient composition. Colour preferences of avian frugivores, in the aviary and in the field, varied among individuals, but the majority favoured red fruits. Seed predators (mostly rodents) did not discriminate between seeds from different morphs. The effect of seed passage through the digestive tract of frugivores (birds and bears) on germination was similar for both morphs, although there were significant differences among frugivores. The type of soil on which the seeds are deposited influenced their germination behaviour, suggesting that some soils could favour one morph over the other. Such differences may contribute to the regional differences in frequencies of the two morphs. This study emphasizes the need to investigate fruit and seed characteristics that correlate with fruit colour; the colour preferences of consumers is only one of several selection pressures that determine the frequency distribution of fruit colours.

Keywords: avian seed dispersal; colour preferences; fruit polymorphism; seed germination; southeast Alaska

Introduction

The study of polymorphisms (e.g. mimicry complexes in butterflies, shell colour in snails) has been very useful in understanding the operation of natural selection. However, some types of polymorphisms, like flower- and fruit-colour polymorphisms, have not received much attention, even though they are widespread in nature. Although a few studies have investigated their ecology (for flowers: Waser and Price, 1981; Kevan, 1983; Ernst, 1987; Stanton *et al.*, 1989; Levin and Brack, 1995; Fry and Rausher, 1997; for fruits: Willson, 1983; Willson and O'Dowd, 1989), we still know little more than Darwin did on this subject, especially on fruit colour. Plants with polymorphic fruits, such as salmonberry (*Rubus spectabilis*), are very suitable for investigating the ecological and evolutionary significance of fruit colour.

One of the most plausible hypotheses explaining the evolution of colour in fleshy fruits is that this trait responds to selective pressures exerted by frugivores (Willson and Whelan, 1990). Frugivores appear to choose fruits at least partly on the basis of colour (Willson *et al.*, 1990; Willson, 1994; Puckey *et al.*, 1996, and references therein), although other factors such as crop size, fruit accessibility and relative abundance are also important in influencing fruit choices. We first investigated the possibility that frugivores contribute to the maintenance of the polymorphism, by performing colour preferences tests in the aviary and examining actual fruit removal rates in the

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field. After describing the assemblage of animals (mostly birds) that consume the fruits of this plant in southeastern Alaska, we asked the following questions: (1) Do frugivorous birds have a colour preference and are there differences in preferences among bird species? (2) Does frequency of a given fruit morph influence its selection by birds?

Alternatively, fruit colour may be determined by selection on correlated characters (Willson and Whelan, 1990), such as different germination behaviours of seeds from different morphs (Willson and O'Dowd, 1989). In addition, seed predators might prey differentially on seeds from different fruit types. Therefore, we also asked: (3) Do frugivores affect the germination behaviour of the two morphs in different ways? (4) Does the type of soil have different effects on the seed germination of the two morphs? (5) Can seed predators discriminate between seeds of red and orange fruits?

Methods

Characteristics of salmonberries

Rubus spectabilis (Rosaceae), commonly known as salmonberry, is a shrub occurring in successional vegetation, gaps and edges of the temperate rainforests of the northwestern coast of North America (from northern California to south-central Alaska). The plant produces ripe fruits of two colours, red and orange (formerly called 'gold'; Willson and Comet, 1993, Willson, 1994). This polymorphism is found throughout the geographical distribution of the species, although the frequencies of the two morphs vary widely (Gervais, 1996). Despite considerable variation among fruits in many traits, the colour morphs of salmonberry fruits are very similar in their general characteristics, although orange fruits tend to be slightly larger (Table 1). The fruit is an aggregate of drupelets that pass through three colour phases during their development, from green when unripe to yellow-orange and finally to red (or orange in the other morph) when mature.

Experiments in the aviary

From 11 July to 11 August 1994, birds were captured in mist-nets at several sites near Juneau, Alaska. Two hand-captured ravens were also used in one experiment. All birds thought to be potential frugivores were kept in an outdoor aviary in Juneau, where they were housed separately in cages $2 \times 2 \times 2$ m, with a long perch along the back wall. The captive birds were initially

Table 1. Characteristics of red and orange colour-morphs of salmonberries (mean ± s.e.; range for macronutrients) collected from different individual plants at different sites near Juneau

Trait	Red morph	n	Orange morph	n	F (or T)	P
Fruit length (cm)	2.21 ± 0.04	75	2.32 ± 0.03	75	1.74	0.02
Fruit width (cm)	2.17 ± 0.03	75	2.22 ± 0.02	75	1.29	0.28
Fruit mass (g)	3.27 ± 0.12	80	3.47 ± 0.12	80	1.08	0.72
No. seeds per fruit	45.8 ± 2.2	20	41.9 ± 1.9	22	1.75	0.18
Seed length (mm)	3.16 ± 0.05	25	2.97 ± 0.06	25	1.15	0.74
Seed width (mm)	1.83 ± 0.05	25	1.67 ± 0.04	25	1.59	0.26
% Lipid (pulp)[a]	1–2%	4	1–2%	5	–	–
% Nitrogen (pulp)[a]	0.5–1%	3	1%	5	–	–
% sugars (pulp)[a]	35–39%	5	33–43%	5	–	–
% Glucose[a]	49–51%	5	49–51%	5	–	–
% Fructose[a]	49–51%	5	49–51%	5	–	–
% Sucrose[a]	Trace	5	Trace	5	–	–

[a] The % nutrients are of dry mass.

presented with salmonberry fruits, other kinds of berries (mainly locally collected *Vaccinium, Ribes* and *Sambucus*) and meal worms; seed eaters were also given sunflower seeds. Once a bird had eaten some fruits (thus establishing its status as a potential frugivore), we started the experiment on colour preferences of salmonberry fruits. The birds were observed from a blind placed inside the aviary. Experiments usually began about 08:00 h and often lasted until mid- or late afternoon. The birds were usually released the same day of capture, but some individuals were kept overnight and released the next day so as to finish the series of experiments. Since the birds were caught at a time when juveniles already had experience of salmonberries, which usually begin ripening by mid- or late June, both juveniles and adults were used for the experiments.

In the first experiment, we presented each bird with a single Petri dish (9 cm in diameter) containing four pieces (20–30 drupelets) of fresh salmonberry, two red and two orange; the dish was placed on the floor of the cage. Because the berries are large, the birds could eat only a few at a time before becoming satiated. Therefore, usually only a half or a third of a berry was offered (but always the same amount of the two morphs), so the birds would keep eating and the experiment could continue. The four pieces of berry were placed at the edge of the dish equidistant (along the perimeter) from adjacent pieces, red alternating with orange. Only the first choice was recorded, and preference was determined as the proportion of choices of a certain colour in 10 trials. After the bird had made a choice, the dish was replaced immediately with another one and rotated so that, if the bird approached from the same side of the cage as the previous trial, it would not encounter the same colour first. Ravens were tested only in Experiment 1.

In the second experiment, the frequency of colours was changed so that the orange fruits were the most common and the red the rarest. Three pieces of orange fruit and one of a red fruit (i.e. 75% orange, 25% red) were presented. The location of the red fruit was rotated 90° for each trial ($n = 10$ trials). The third experiment was like the second, but the frequency was reversed, so the bird was offered three pieces of red fruit and one piece of an orange fruit.

Fruit removal in the field

From 12 sites near Juneau, we recorded the frequencies of each colour morph by counting a minimum of 500 fruits per site (except in sites where salmonberries were not abundant). The fruits were counted from haphazardly chosen shrubs in transects or along trails. Ripe fruits and recently removed fruits (distinguished by the fresh receptacle) were recorded to determine the removal rate of the two fruit morphs in the field. Fruits were counted near the beginning of the fruit ripening season (3–6 July) at nine sites and at the end of it (1–20 August) at eight sites. We were able to examine five of the sites in both periods, because of a great fruit abundance and fruiting asynchrony among individuals.

The two morphs do not differ in fruit-crop size (Gervais, 1996). The plant has extensive vegetative propagation (Tappeiner *et al.*, 1991) and it is often difficult to discriminate among individuals.

Seed germination tests

Rubus seeds of the two colour morphs were obtained in the aviary from droppings of three individuals of American robins (*Turdus migratorius*) and three varied thrushes (*Ixoreus naevius*), separately. In addition, red and orange salmonberries were separately fed to brown and black bears at the zoo in Anchorage, Alaska, and the seeds were extracted later from their faeces. We planted 500 seeds of each morph from each treatment in potting soil in August 1993 (100 seeds per pot, five pots per treatment). All pots were randomly placed in a tray (91 × 61 cm), which was covered with a lid of 1 cm wire mesh to prevent seed predation by rodents. The tray was placed on the forest floor and surrounded by a fence to reduce possible animal disturbances. We recorded seed

germinations periodically from 3 May 1994 until 15 July 1995. All germinated seeds were removed from the pots when they were recorded.

To determine whether soil type had an effect on the seeds of the two colour morphs, we collected soil from two areas with differing frequencies of colour morphs. In Sheep Creek Valley, most (66%) of the fruits are orange, whereas near Amalga Harbor, 63% of the fruits are red. The Sheep Creek site is dominated by *R. spectabilis*, cottonwood (*Populus trichocarpa*), alder (*Alnus sitchensis*) and willow (*Salix* spp.), whereas the Amalga Harbor site is a long, narrow clearcut, mainly dominated by *Vaccinium* spp., *Sambucus racemosa*, *Oplopanax horridum*, several species of ferns and seedlings of hemlock (*Tsuga heterophylla*), but also with abundant salmonberry. On 29 July 1994, we filled 60 pots with three different types of soil: in 20 we placed soil collected under salmonberry from Sheep Creek; in another 20 we placed soil gathered below salmonberry plants at Amalga Harbor; the last 20 pots were filled with potting soil and used as controls. We planted 100 seeds of red fruits per pot in half of the pots of each treatment and 100 seeds of orange fruits per pot in the remaining half. Seeds were obtained from fruits collected at several locations. All pots (9 cm square) were randomly assorted in a tray, and fenced, as described above. For this experiment, the tray was placed in a sunny site near the edge of the forest (at the site called Two-mile). Germination was recorded periodically, from 2 May until 15 July 1995.

Seed predation by rodents

Seed predation by rodents was assessed in four of the sites used to record the frequencies of each colour morph. Along a transect or a trail in each area, and separated by approximately 60 m from each other, we placed 10 rectangular pieces of wood on the extremes of which we nailed two aluminium dishes (5 cm in diameter), separated by about 15 cm. The tables were hidden underneath salmonberry or other shrubs. Twenty seeds of each morph were separately placed on the two dishes of each table. The experiment was set in all sites on two consecutive days. Seed removal was checked after 5 days, recording the number of seeds remaining of each morph. Five trials were conducted at each site. The experiment began on 4 July and ended on 1 August 1995. All seeds were replaced with new ones for each new trial.

Data analyses

Chi-square was used to determine fruit preferences in the aviary experiments. The frequencies of each morph and the proportions of fruits removed in the field were compared using Wilcoxon's test. Various repeated-measures analyses of variance (RMANOVA hereafter) were performed, with the general linear model (GLM) procedure in SAS (1987), to examine differences in seed germination patterns of the two morphs. A two-way analysis of variance tested for differences in seed predation between the two morphs and among sites. Both the angular transformation and the square-root transformation were used to normalize the data before analysis.

Results

Frugivores and preferences

A variety of animals consume the fruits of *R. spectabilis* (Table 2), and most are 'legitimate' seed dispersers of the plant (i.e. swallow the fruits and pass the seeds intact). Although red squirrels are important seed predators, they can also disperse seeds by dropping partially eaten fruits. Both brown and black bears are important consumers of salmonberry. Although they are effective agents of seed dispersal, their droppings usually contain thousands of seeds and the seedlings are subject to

Table 2. Known consumers of *Rubus spectabilis* fruits in southeast Alaska, indicating whether they act as seed dispersers or seed predators (or unknown)

| Class | Species | | Disperser category |
	Common name	Scientific name	
Birds	Common raven	*Corvus corax*	LD
	Northwestern crow	*Corvus caurinus*	LD
	Steller's jay	*Cyanositta stelleri*	LD
	American robin	*Turdus migratorius*	LD
	Swainson's thrush	*Catharus swainsoni*	LD
	Hermit thrush	*Catharus guttatus*	LD
	Varied thrush	*Ixoreus naevius*	LD
	Fox sparrow	*Passerella iliaca*	ND
	Lincoln sparrow	*Melospiza lincolni*	ND
	Dark-eyed junco	*Junco hyemalis*	ND
Mammals	Brown bear	*Ursus arctos*	LD
	Black bear	*Ursus americana*	LD
	Red squirrel	*Tamiasciurus hudsonicus*	ND?
	Black-tailed deer	*Odocoileus hemionus*	??
	Pine marten	*Martes americana*	LD
Mollusca	Banana slug	*Ariolimax columbianus*	LD

LD, legitimate dispersers; ND, non-legitimate dispersers. All bird species were tested in the aviary except Steller's jay; data for crows in Willson and Comet (1993). Seeds of *R. spectabilis* have also been reported from salmon and charr stomachs in southeast Alaska by sport-fishing biologists.

severe competition and mortality (M.F. Willson, unpublished). Banana slugs also consume salmonberry fruits and disperse seeds short distances (Gervais *et al.*, in press).

The experiments performed at the aviary showed that 16 of the 58 individuals tested (28%) had no significant preference for either fruit morph, while the remaining 42 (72%) showed significant preferences ($P < 0.05$, χ^2 tests), mostly (77%) for the red morph. There was individual variation in most species, but overall the red morph was selected more frequently than the orange one ($\chi^2 = 13.7$, $P < 0.01$; Table 3). Such variation was observed as much in adult birds as in juveniles, which already had experience of the fruits in the field.

There was some evidence of frequency-dependent colour choice in the aviary experiments. When red was more frequent, significantly more individuals (of those that had a significant preference) selected red than orange ($\chi^2 = 5.4$, $P < 0.05$). When orange was most frequent, that colour was favoured slightly, but not significantly, more often ($\chi^2 = 1.6$, N.S.) (Table 4). However, about a third of the birds showed no significant preference for either morph, and fewer than half the birds favoured the more common morph in Experiment 2 or Experiment 3. Thus, there was little evidence of a general tendency for fruit choices in the aviary to be frequency-dependent, although some birds did select the more common morph.

Twenty-three of 56 individuals (41%) made consistent choices in all three experiments: 16 (14 thrushes and 2 sparrows) always preferred red, 3 (2 thrushes and 1 sparrow) always preferred orange, regardless of the frequency of each morph, and 4 birds (all fox sparrows) consistently showed no preference for either colour. Such consistencies were observed both in adult and juvenile birds. The tendency to shift to the more common fruit colour did not differ between the two morphs ($\chi^2 = 0.007$, $P = 0.9$) (Table 4).

Table 3. Number of individuals that showed the indicated preference in aviary trials[a]

Species	n	Red	Orange	No preference
Common raven	2	2	0	0
American robin	4	1	1	2
Swainson's thrush	16	10	3	3
Hermit thrush	13	10	3	0
Varied thrush	6	3	0	3
Fox sparrow	13	5	2	6
Lincoln sparrow	2	1	0	1
Dark-eyed junco	2	1	0	1

[a] A preference for a colour morph was defined as a choice for colour in at least 8 of 10 trials (or in 5 of 5 trials in the few cases when no more trials were possible). n = number of individuals of that species tested.

Table 4. Fruit preferences by birds when the colour frequencies were 3:1[a]

Frequencies	n	Red morph	Orange morph	No preference
3O:1R	32	8 (25%)	14 (44%)	10 (31%)
1O:3R	26	12 (46%)	3 (12%)	11 (42%)

[a] The different sample sizes are due to the lack of 'cooperation' by some birds to finish the three experiments. Figures in parentheses represent percentages of the individuals tested at each frequency distribution of colour morphs. O, orange; R, red.

Table 5. Fruit-colour preferences by 'legitimate' avian seed dispersers (thrushes) versus 'non-legitimate' dispersers (juncos and sparrows) in the first experiment (50% red, 50% orange)[a]

Disperser type	n	Red	Orange	No preference
Legitimate	39	20 (51%)	6 (15%)	13 (33%)
Non-legitimate	17	4 (24%)	2 (12%)	11 (65%)

[a] Figures in parentheses represent percentages of the individuals tested from each group.

If colour preferences of avian dispersal agents have exerted an important selection pressure for fruit colour, fruit-colour preferences might be stronger in good dispersal agents than in poor ones. To test this, we divided the bird species tested (except ravens) into two groups: 'legitimate' dispersers (all the thrushes) and 'non-legitimate' dispersers (those that usually eat the pulp and discard or eat the seeds; sparrows and juncos). Thrushes chose red fruits marginally more often than sparrows and juncos ($\chi^2 = 4.95$, $P = 0.08$; Table 5). However, consistency in preference for red fruits across all three experiments did not differ significantly between groups: 2 of 17 'non-legitimate' dispersers consistently chose red, whereas 14 of 39 thrushes did so ($\chi^2 = 2.30$, $P = 0.13$).

In the field, the ratios of the two fruit morphs varied significantly among sites (Table 6), but, on average, they were about equally common. This was so both early and late in the fruiting season (Wilcoxon's test, $\chi^2 = 0.93$, $P = 0.34$). Fruit removal, relative to the total number of fruits (red and orange) available, did not differ significantly between morphs ($\chi^2 = 1.80$, $P = 0.17$) (Table 6). Fruit removal was frequency-dependent (i.e. a higher proportion of either morph was removed when it was more frequent in an area) in the first part of the fruiting season ($r_s = 0.87$, $P = 0.003$

and $r_s = 0.67$, $P = 0.05$, for red and orange berries, respectively) (Fig. 1). The number of fruits removed relative to the number available of each morph was also similar between morphs ($\chi^2 = 0.83$, $P = 0.36$). Possibly due to an increase in availability of ripe fruits of other species (*Vaccinium*, *Sambucus*, *Ribes*, *Streptopus*, etc.), removal of salmonberries decreased with time.

Table 6. Ratio of the frequencies of the two fruit-colour morphs available and of removal by birds in different sites at the early and late fruiting season[a]

Site	Early season		Late season	
	Availability R:O	Removal R:O	Availability R:O	Removal R:O
Two-mile	0.49	0.48	—	—
Thane Road	0.90	1.26	—	—
Perseverance	2.45	3.63	2.06	1.63
Granite Basin 1	0.61	0.86	2.32	3.27
Granite Basin 2	—	—	4.71	0.001
Granite Basin 3	—	—	1.01	2.75
Sheep Creek 1	4.97	6.49	1.20	1.50
Sheep Creek 2	0.52	0.42	1.00	1.25
Blueberry Hill	2.70	5.69	—	—
Mt. Roberts	0.15	0.20	0.63	1.33
Amalga	1.71	2.13	—	—
Montana Creek	—	—	2.69	3.56

[a] Frequency of each morph (availability) was obtained by adding together the ripe fruits present and the fresh receptacles from which fruits had recently been removed. O, orange; R, red.
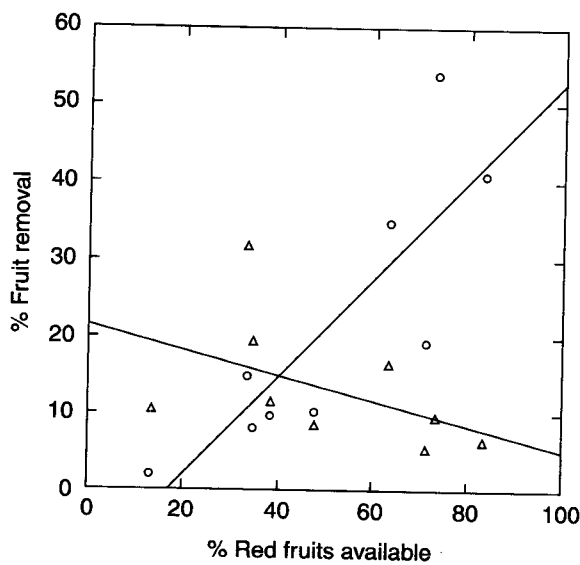

Figure 1. Relationship between frequency of each morph and proportion of fruits removed in the wild during the first part of the fruiting season (see r^2 in text). Fruit removal late in the season decreased notably and there was no frequency-dependence (see text). △, orange morph; ○, red morph.

Removal of red berries (relative to total fruit availability) decreased from $21.6 \pm 5.9\%$ (mean $\pm$ s.e.) to $7.7 \pm 2.3\%$ ($\chi^2 = 3.70$, $P = 0.05$), while removal of the orange ones declined from $13.2 \pm 2.8\%$ to $3.9 \pm 1.2\%$ ($\chi^2 = 7.79$, $P = 0.005$). When only the five sites examined both in July and August were considered, fruit removal of either morph was observed to decrease by half in the second part of the fruiting season (from 16 to 8% in the red morph, and from 11 to 5% in the orange morph).

Seed germination behaviour

Effect of seed dispersers. Germination patterns of salmonberry seeds varied with treatment in the digestive tracts of different frugivores but not between morphs in the overall RMANOVA (treatment $F_{4,40} = 3.46$, $P = 0.02$; morph $F_{1,40} = 1.18$, $P = 0.29$; interaction not significant; Wilks' lambda $= 0.08$, $F = 15.63$, $P < 0.001$ for the time effect; all interactions with time not significant). For both morphs, passage through a consumer's gut enhanced germination (Fig. 2). Throughout much of the test period, for the orange morph, the temporal pattern of difference between treatment and control varied among frugivores: seeds passed by black bears and by varied thrushes differed significantly from the control only at the beginning, whereas those passed by robins and brown bears differed from the control for much longer. In separate RMANOVAs comparing the behaviour of the two morphs within a treatment, differences between morphs were found only for the robin ($F_{1,8} = 7.99$, $P = 0.02$); germination rate was higher in the orange than in the red morph after the seeds passed through robins' guts (Fig. 2). However, the final number of germinated seeds was similar in all treatments ($F_{4,40} = 1.46$, $P = 0.23$) and in the two morphs ($F_{1,40} = 1.48$, $P = 0.23$), and there was no significant interaction (Fig. 2).

Effect of soil type. Seed germination patterns differed significantly depending upon the type of soil on which the seeds were planted ($F_{2,54} = 15.10$, $P = 0.0001$) but were similar between the two morphs in the overall RMANOVA ($F_{1,54} = 1.43$, $P = 0.24$), even though the effect of treatment was not the same for the two morphs (interaction $F_{1,54} = 3.61$, $P = 0.03$) (Fig. 3; Wilks' lambda $= 0.11$, 0.26 and 0.58; $F = 55.02, 6.64$ and 2.17, $P \leq 0.01$, for the time effect, time $\times$ treatment, and time $\times$ morph $\times$ treatment, respectively; time $\times$ morph not significant). Both morphs germinated better in Amalga soil than in Sheep Creek soil, but the difference between morphs was much greater in Sheep Creek soil than in Amalga soil (Tukey's test). The mean proportion of seeds that germinated was similar between 'Amalga' and 'control' (37.4% and 40.4%, respectively) but was lower in the 'Sheep Creek' soil (24.8%). In this latter treatment, an average of 30.5% of the seeds of the orange morph had germinated by the end of the experiment, whereas only 19.1% of the red morph had done so (Fig. 3).

Seed predation

The animals that potentially prey on seeds in the area are rodents (squirrels, mice and voles) and ground-foraging birds. They did not appear to discriminate between seeds that come from red salmonberries and those that come from orange ones ($F_{1,382} = 0.04$, $P = 0.85$; Fig. 4). Seed removal varied among sites ($F_{3,382} = 46.92$, $P < 0.001$), being significantly greater at Two-mile and Perseverance ($67.3 \pm 3.5\%$ and $54.0 \pm 4.2\%$, respectively) than at Amalga or Sheep Creek ($18.0 \pm 2.9\%$ and $15.6 \pm 2.7\%$, respectively), and no association was found between morph and location ($F_{3,382} = 0.29$, $P = 0.83$).
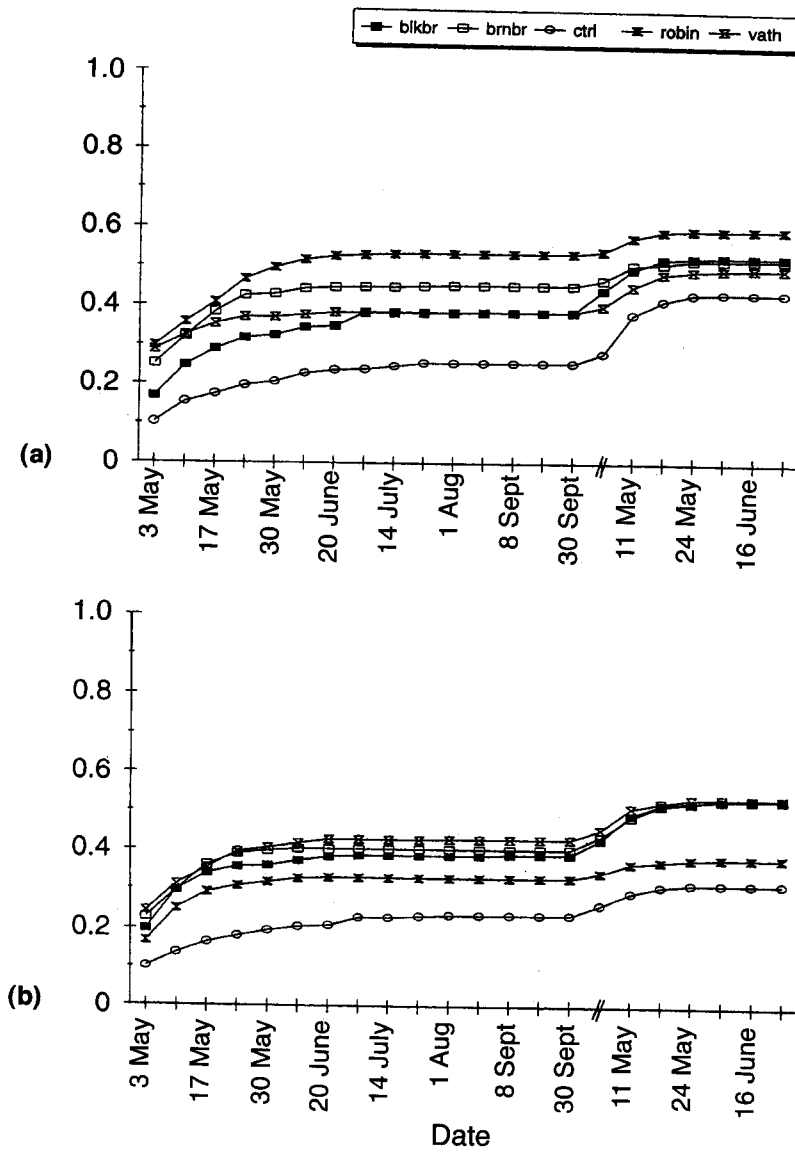
Figure 2. Proportion of seeds of the orange (a) and red (b) morph that germinate after passing through the digestive system of black bears (blkbr), brown bears (brnbr), robins (amro) and varied thrushes (vath) and of control (ctrl) seeds not passed through any animal. Germination was recorded from 3 May 1994 until 15 July 1995.
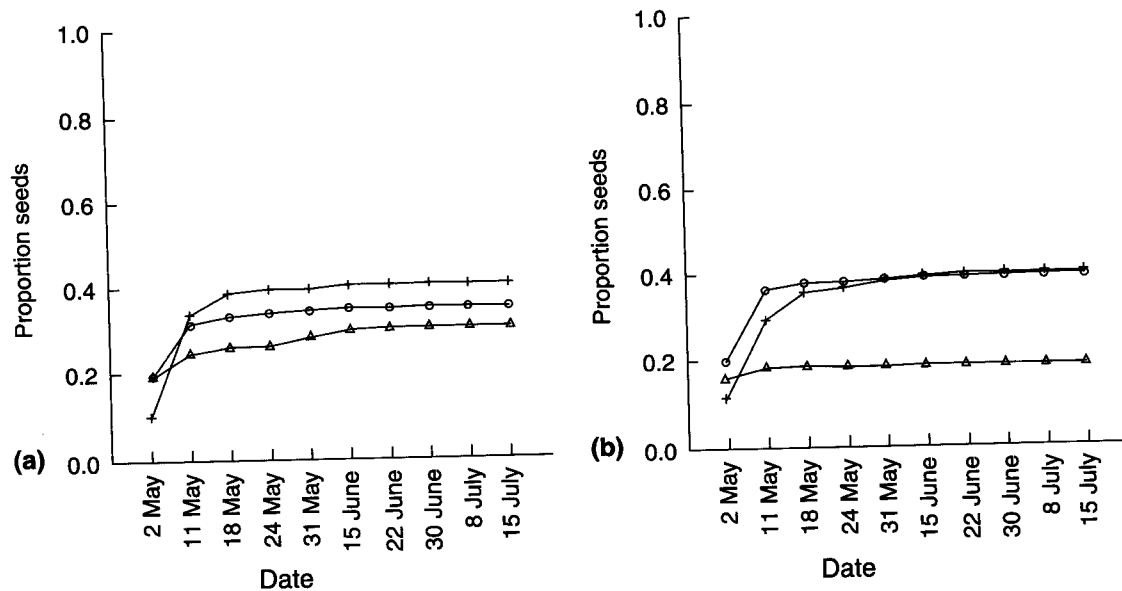
Figure 3. Cumulative proportion of seeds per pot of the orange (a) and red (b) morph that germinate in different types of soil: O, soil from Amalga, where 63% of the fruits are red; △, soil from Sheep Creek 2, where only 34% of the fruits are red; +, pot soil (control).
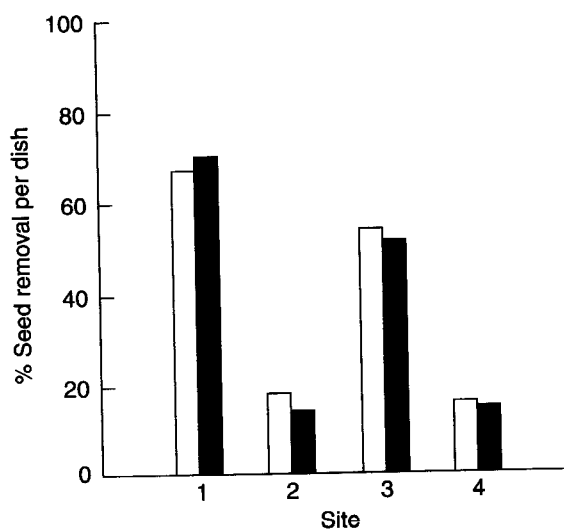

Figure 4. Mean number of seeds of the two morphs that remained in the experimental dishes after 5 days of being exposed to seed predators at four different sites. A total of 20 seeds of each morph were originally placed in each dish. Five trials were conducted at each site. 1, Two-mile; 2, Amalga Harbor; 3, Perseverance; 4, Sheep Creek. ■, orange; □, red.

Discussion

The role of frugivores as selective agents

Of all measured fruit traits with potential importance to bird consumers, the only marked difference between the two morphs of salmonberry was colour. Most birds that feed on salmonberries in the area preferred red fruits, although there was great variation both within and among species. Even though red is the most common colour of fleshy fruits consumed by birds, and some individuals show strong preferences for this colour (McPherson, 1988; Willson *et al.*, 1990; Willson and Comet, 1993; Willson, 1994; Puckey *et al.*, 1996; this study; but see Murray *et al.*, 1993), in most cases the preferences are variable and labile (e.g. McPherson, 1988; Willson and Whelan, 1990; Willson *et al.*, 1990; Willson and Comet, 1993; Willson, 1994). In this study, less than half of the birds tested made consistent choices in all three experiments. Likewise, Northwestern crows often preferred red salmonberries but sometimes favoured orange or showed no preference (Willson and Comet, 1993). A parallel situation occurs in flower-visiting birds: although many hummingbird flowers are red, hummingbirds do not always prefer red (Grant and Grant, 1968; Waser and Price, 1981) and can be readily trained to visit flowers of other colours (Grant and Grant, 1968; Stiles, 1976; Goldsmith and Goldsmith, 1979).

The high individual variability of preferences for the salmonberry colour morphs found in this study and others (Willson and Comet, 1993; Willson, 1994) may reflect the high frequencies of both colour morphs in southeast Alaska. However, preliminary experiments with robins on colour preferences of two other polymorphic species of this region (*Sambucus racemosa* and *Streptopus streptopoides*), both with a much greater frequency of the red morph than the orange one, also showed individual variation; overall, there was no preference for red or orange berries of either species, although adult robins appeared to prefer red over orange berries of *Sambucus racemosa* (Willson, 1994). 'Legitimate' dispersers (thrushes) of *R. spectabilis* tended to choose red fruits more often than 'non-legitimate' dispersers (sparrows and juncos), when given equal frequencies of red and orange berries (test 1). That thrushes are important frugivores and thus are more familiar with the red fruits of most species than are sparrows and juncos (mainly insectivores and seed-eaters) might account for such slight differences. The consistency in fruit preferences across the three tests was, however, equally variable between the two groups, suggesting that the former do not exert a more relevant selective pressure on fruit colour, if any, than the second group.

'Experience' with one colour morph might affect future colour choices, which must be considered when looking at the outcomes of aviary experiments (Willson and Comet, 1993). In our experiments, the greater exposure of birds to the orange morph in the second test (orange:red = 3:1) did not result in a preference for this morph in the third test, indicating that immediate experience with one colour was not an important factor determining later fruit choice, at least for most individuals. Likewise, experience was not a good explanation for the colour preferences of Northwestern crows; hand-raised crows did not prefer the colour of the food upon which they were raised (Willson and Comet, 1993). Furthermore, in silvereyes (*Zosterops lateralis*) in Australia, exposure to a maintenance diet of a particular colour failed to alter bird preference for the red fruits of *Rhagodia parabolica* (Puckey *et al.*, 1996).

Differences in handling time have been suggested to be the main reason why hummingbirds discriminate between blue and white flowers of *Delphinium nelsonii* (Waser and Price, 1981). However, there were no detectable differences in handling time for fruits of salmonberry. Both red and orange salmonberries are easily detached from the receptacles once mature, fruits and drupelets are about the same size, and they contain similar nutrient contents and seed loads. Direct observations in the aviary further indicated that birds spend the same amount of time with both fruit morphs.

Results obtained with captive animals may not always reflect conditions in the wild (e.g. McPherson, 1988; Willson and Comet, 1993), as other factors, such as fruit abundance, spatial distribution, accessibility and fruit conspicuousness against foliage, may determine fruit choices by frugivores. However, both red and orange salmonberries seem to be similarly conspicuous (detectable) against the green foliage of the shrubs (see Lee *et al.*, 1994), to human eyes at least, so if there is an effect of conspicuousness on fruit discovery rate, it may not differ much between morphs. The removal of salmonberries in natural conditions did not differ between morphs overall (although in experimental plots, red fruits were depleted first; Gervais, 1996). Removal was frequency-dependent in the field, at least in the first part of the fruiting season (when most fruit removal takes place). In the field, familiarity with a given colour morph in an area (because of their greater abundance) might actually affect the preference for that colour at that particular site; birds within an area might form a kind of temporary search image for fruits of the more common colour (Willson, 1994).

Human berry-pickers commonly prefer the red fruit-morph. However, five test humans who stated a preference for red salmonberries over orange ones (after having tasted them in the field) could not distinguish between the two morphs when they were blindfolded. The flavour of salmonberries varies greatly, to human tastes, but much of the variation is found within, not between, morphs. One possible cause of preferences for the red morph, by both humans and birds, may be that red is often a cue that indicates fruit ripeness. Salmonberries of the red morph have a hue between yellow and orange just before reaching maturity. It is thus possible that some birds associate the orange colour with immaturity of salmonberries. In the aviary, some individuals that had chosen red fruits in the first experiment (and even had chosen the less frequent red berries in some trials of the second experiment) eventually happened to peck an orange berry and from then on continued to choose this morph, doing so even in the third test (when orange was less frequent), a preference shift also observed by Willson (1994). These aviary observations suggest that at least some birds may have to learn that orange fruits are edible. Frequency-dependent fruit removal in the field suggests that many birds learn sooner or later that orange salmonberries are also edible and nutritious.

The finding that fruit removal in natural conditions (all sites pooled) does not differ significantly between the two morphs bolsters the idea that avian frugivores, at least through their colour preferences, do not exert strong present-day selection pressures on fruit colour (Willson and Whelan, 1990; Willson and Comet, 1993; Willson, 1994). Thus, avian foraging behaviour may help maintain the colour polymorphism.

The response of seeds to passage through different kinds of dispersers (birds and bears) differed little between the two morphs. Only seeds of the orange morph showed a slight shift in germination behaviour after passing through the digestive tract of robins, but whether such enhanced germination enables the orange morph to respond faster than the red morph to favourable conditions is not known. In any case, it seems unlikely that differences in seed responses to treatment in vertebrate digestive tracts contributes to the maintenance of the colour polymorphism in the population. These results contrast with those from *Rhagodia parabolica*, in which seeds of the white morph showed a greater response to passage through silvereyes' guts than the red or the yellow morph (Willson and O'Dowd, 1989).

The seeds of the two morphs germinated faster after passing through an animal's digestive system than unpassed seeds. Such germination enhancement has been reported in many studies (e.g. Willson, 1983; Lieberman and Lieberman, 1986; Barnea *et al.*, 1990, 1991; Izhaki and Safriel, 1990; Midya and Brahmachary, 1991; Clergeau, 1992; Murphy *et al.*, 1993; De Figueiredo and Perin, 1995; Traveset and Willson, 1997) and is often due to a modification of the seed coat that increases permeability and allows gas exchange and water intake (Barnea *et al.*, 1990; Izhaki and

Safriel, 1990). Differences among animals have sometimes been attributed to variation in seed-retention times in digestive systems, with longer periods causing a stronger effect on seed abrasion (Barnea *et al.*, 1990, 1991; Izhaki and Safriel, 1990; Clergeau, 1992; but see Murphy *et al.*, 1993). However, the differences observed between dispersers with similar seed-retention times (black and brown bears; robins and varied thrushes) suggest that other, unknown, factors besides retention times can affect germination rate. Most germinations, as observed in other species in the area (Traveset and Willson, 1997), took place during spring after planting (see Fig. 2). A similar percentage of the seeds (treated or not) were found to have germinated by the end of the experiment, indicating that passage through an animal's gut does not affect seed mortality.

Effect of soil type on seed germination

The source of soil in which the seeds were planted had a significant effect on seed germination. Seeds of the two morphs germinated at the same rate in Amalga and in potting soil but at a lower rate in Sheep Creek soil. The orange morph, the most common in Sheep Creek, germinated more rapidly than the red morph in soil from this site. Some edaphic characteristics are known to inhibit seed germination, modifying osmotic pressure (Evenari, 1949; Mayer and Poljakoff-Mayber, 1975; Debussche, 1985) and affecting the metabolic processes secondarily related to pigments of both flowers (Horowitz, 1976) and fruits (Chenery, 1948). Furthermore, soil-borne pathogens can kill the embryos of some seeds (Titus *et al.*, 1990). A more detailed study is needed to ascertain what soil components and pathogens can affect seed germination in this species, and by what mechanisms.

Seed predators as selective agents

Animals that prey upon the seeds of *R. spectabilis* did not discriminate between the seeds of the two colour fruits at any of the four study sites examined. This indicates that seed predators are quite unlikely to exert any selective pressure for either colour morph or for the maintenance of the polymorphism. No differences in consumption of white and red fruits, or their seeds, by pre- and post-dispersal predators were observed in the only other species, *Actaea rubra* (Ranunculaceae), in which this has been examined (Willson, 1983)

Evolution and maintenance of the polymorphism

Colour differences can arise from relatively simple mechanisms, such as the attachment of a particular molecule to a pigment precursor (see Willson and Whelan, 1990 and references therein), and spread by gene flow (Levin and Brack, 1995). Two other species in southeast Alaska produce both red and orange fruits, but the frequency of the orange morph is much lower than in salmonberry: ≪1% in elderberry (*Sambucus racemosa*; Willson, 1994), 0–9% in five populations of small twisted stalk (*Streptopus streptopoides*; A. Traveset and M.F. Willson, personal observations).

In contrast, the orange morph of salmonberry is very common in southeast Alaska and far more common than the red morph in the southern end of its geographic range (Gervais, 1996). It is difficult to say, at this point, which colour is the 'original' colour and which is the 'mutant' form. The differences we observed in seed germination behaviour on different soils suggest the hypothesis that the distribution of soil types suitable for germination and available for colonization may contribute to regional differences in frequency. In any case, it is clear that simple colour preferences of consumers are unlikely to account for the frequency distribution of fruit colours, and that other factors need to be investigated.

Acknowledgements

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