SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ANIMAL BEHAVIOUR
BEHAVIOURAL ECOLOGY

Received
1 November 2013

Accepted
18 June 2014

Published
17 July 2014

Correspondence and requests for materials should be addressed to R.-C.Q. (quanrc@xtbg.ac.cn)

Bird fruit preferences match the frequency of fruit colours in tropical Asia

Qiong Duan[1], Eben Goodale[2] & Rui-chang Quan[1]

[1]Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan, 666303, China, [2]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

While many factors explain the colour of fleshy fruits, it is thought that black and red fruits are common in part because frugivorous birds prefer these colours. We examined this still controversial hypothesis at a tropical Asian field site, using artificial fruits, fresh fruits, four wild-caught resident frugivorous bird species, and hand-raised naïve birds from three of the same species. We demonstrate that all birds favored red artificial fruits more than yellow, blue, black and green, although the artificial black colour was found subsequently to be similar to the artificial blue colour in its spectral reflectance. Wild-caught birds preferred both black and red fleshy natural fruits, whereas hand-raised naïve birds preferred black to red natural fleshy fruits and to those of other colours. All birds avoided artificial and naturally ripe green fruits. The inter-individual variation in colour choice was low and the preferences were constant over time, supporting the hypothesis that bird colour preferences are a contributing factor driving fruit colour evolution in tropical Asia.

Globally, the most common colours of bird-dispersed fleshy fruit are red and black[1–4]. Other fruit colours occur at lower frequencies and are dispersed chiefly by non-avian frugivores[1,5]. Fruit colours are commonly considered a key feature in attracting avian dispersers because birds have excellent colour vision systems and probably use colour to locate fruits. As such, the frequency of fruit colours in many habitats may be the evolutionary result of the differential foraging preference of birds for these colours. In other words, it has been hypothesized that fruit colours are adaptive in attracting bird frugivores[6] (and references therein [6]). This traditional hypothesis has recently been strengthened by some studies indicating that fruit colour and trait diversity can be well explained by their animal consumers[7–9], more so than by phylogenetic relationships among plants[10]. In addition to color, conspicuousness is also an important visual signal affecting bird food preferences, and red and black fruit are generally more conspicuous than other colours[4,11–14].

Although red and black are the fruits dispersed chiefly by birds, it is still contentious as to whether the frequency of fruit colours, especially black and red, in the wild match the colour preferences of bird consumers. For example, some studies have shown that birds select fruit of a certain colour, but that colour was not necessarily red or black[15,16]. Other studies found that birds prefer red fruit[17–19], but there is wide individual variation in fruit-colour preference[12,16,19]. These inconsistencies in colour preferences suggest that birds may not provide strong and constant positive selection for red and black fruit. It should be remembered that other factors, such as phylogenetic inertia of plants[20] and other functions of pigment in fruit may also influence fruit colours. For example, the important pigment class of anthocyanins may play multiple roles in protecting vegetative tissue and fruit and reducing fungal growth[21]. Further, the selection of fruit by birds may be driven by factors other than colour: traits like nutritional contents and secondary compounds also influence bird food preference[6,21]. For example, birds may actually prefer anthocyanins because of their antioxidative properties[22]. As nutritional contents and colour often positively covary in bird-consumed fruits and birds are visually guided foragers, frugivorous birds can use colour as indicators of nutritional rewards[9].

Experience is another important aspect of birds' fruit colour preference[23–25]. In the wild, early experiences of food items may be influenced by social learning[26–28]. These early experiences may help young birds to identify favourable food sources and avoid unfavourable food sources later in life. In some species, the effect of early social learning on aspects of food lasts for life[28]. Thus hand-raised naïve birds are advantageous for determining the influence of experience on food selection by fruit-eating birds because, unlike wild-caught conspecific birds, their colour preferences have not been influenced by previous experience. If colour preference is innate, hand-raised birds are expected to prefer fruits of the most common colour in their environments. This is because common fruit colours (e.g., red) can facilitate the detection and recognition of food sources by inexperienced birds[25,29]. Therefore, one important aspect for understanding whether the frequency of fruit colour is influenced by the preferences of frugivorous birds is to determine whether innate preferences match common fruit colours. Although this question has been addressed in some studies by comparing the colour preference of wild-caught animals to hand-raised naïve animals (e.g.[24,25,30]), the extent to which both learnt and innate colour preferences reflect the frequency of fruit colour in nature has not yet been clearly established. Furthermore, in the few studies that tested naïve colour preference, there was strong inter-individual variation (e.g.[30]). In the absence of a stable pattern, naïve preferences do not seem likely to be related to the frequency of fruit colour. Therefore, more studies are needed to improve our knowledge of the colour preferences of bird dispersers, especially using hand-reared birds.

Tropical Asia is an ideal location for expansion of our knowledge of the colour preferences of birds because tropical Asia has the largest diversity of frugivorous bird species in the world[31]. These birds also play important roles in the seed dispersal of tropical Asia[32,33]. However, very few studies have been conducted in this region (but see Duan and Quan[34]). In this study, we experimentally addressed several questions concerning the preferences of frugivorous birds. Four bird frugivores from tropical Xishuangbanna, southwest China, were used in this experiment, selected because of their abundance at the study site and because they are representative of two important families of Asian frugivores (bulbuls and barbets)[31]. The following questions were addressed: (1) Do naïve hand-raised birds prefer fruits of a certain colour, in a way similar to wild-caught birds? Does this preferred colour correspond to the frequency of ripe fleshy fruit colours in this area? It was hypothesized that black and red fleshy fruits would be preferred by such hand-raised birds, as well as wild-caught ones. (2) Does experience influence the colour preferences of birds for fruit? Do the colour preferences of experienced or naïve birds better reflect local frequencies of fruit colour? We hypothesized that the colour preferences of naïve birds would be closest to the frequency of fruit colour in the wild. (3) Are the colour preferences of both experienced and naïve birds constant over time (specifically over several days of repeated trials)? We used the following colours for testing these hypotheses on artificial and natural fruit: black, blue, green, red and yellow; however, note that the artificial black colour was found subsequently to be close to the artificial blue colour in its spectral reflectances[34] (see discussion).

Results

Artificial fruit colour selection. Juvenile birds of all species differed in colour preference of artificial fruit from what would be expected randomly (ANOVA, $F_{4,\,555} = 639.1$, 117.5 for *Pycnonotus jocosus*, *P. aurigaster* respectively, and $F_{4,\,380} = 79.3$ for *Megalaima asiatica*; $P < 0.001$ for all). Hand-raised *P. jocosus*, *P. aurigaster*, and *M. asiatica* (14, 14, and 9 individuals, respectively) all preferred red artificial fruits over artificial fruits of other colours (Figure 1A).

Wild-caught *P. jocosus*, *P. aurigaster*, and *P. melanicterus* (10, 5, and 7 individuals, respectively) also showed a strong difference in colour preference for the artificial fruits (ANOVA, $F_{4,\,295} = 74.4$, $F_{4,\,160} = 22.2$, and $F_{4,\,235} = 47.8$ respectively; $P < 0.001$ for all) with red fruits consistently preferred over fruits of any other colour (Fig. 1B). Wild-caught barbets did not eat any artificially coloured fruit during the experiment.

There was weak inter-individual variation in the artificial fruit colour preference among hand-raised juveniles (Appendix S1-1). All 14 juvenile *P. jocosus* showed a consistent preference for red fruits over fruits of other colours. However, only 11 of the 14 juvenile *P. aurigaster* and 7 of the 9 juvenile *M. asiatica* preferred red fruits over fruits of other colours. Approximately 86% (32/37) of all hand-raised birds showed a significant preference for red fruits.

There was also weak inter-individual variation in the artificial fruit colour preference of wild-caught adult birds. All 10 *P. jocosus*, four of the five *P. aurigaster*, and six of the seven *P. melanicterus* individuals showed a significant preference for red fruits (Appendix S2-1). In total, about 90% (19/21) of the wild-caught individuals had a significant preference for red artificial fruits.

Natural ripe fruit colour selection. Hand-raised juveniles also showed a strong difference in colour preference for the fleshy fruits from what would be expected randomly (ANOVA, $F_{4,\,535} = 139.6$, $F_{4,\,455} = 298.4$, $F_{4,\,370} = 78.7$ for hand-raised *P. jocosus*, *P. aurigaster*, and *M. asiatica*, respectively, $P < 0.001$ for all). Juveniles of all species preferred black fruits to red fruits and ripe fruits of other colours (ANOVA followed by multiple Bonferroni-corrected two sample T-tests, $P < 0.01$ for black *vs.* red, black *vs.* yellow, black *vs.* green, and black *vs.* blue). In general, black fruits were always the most preferred and ripe green fruits were the least preferred for all species of hand-raised juveniles (Fig. 2A). It is important to recognize that the juveniles' preferences may have been influenced by nutritional differences among the fruits, once they had consumed them. However, if one only considers the first fruit an individual consumed, the pattern remain similar: 16 of the 31 hand-raised juveniles first ate black fruits, with red fruits being the second most consumed category (8 individuals) (Appendix S3).

Wild-caught adults also showed a strong colour preferences for fleshy fruits, different than what would be expected by chance ($F_{4,\,390} = 55.6$, $F_{4,\,135} = 14.5$, $F_{4,\,250} = 44.7$, and $F_{4,\,130} = 12.6$ for wild-caught *P. jocosus*, *P. aurigaster*, *P. melanicterus*, and *M. asiatica*, respectively; $P < 0.001$ for all). However, with the exception of *P. jocosus*, which preferred red over other colours, wild-caught adults showed a significant preference for both red and black fruits over fleshy fruits of other colours. For example, *M. asiatica* and *P. melanicterus* adults showed no difference in preference for flesh black and red ($P > 0.5$), but they preferred black/red fruits to yellow, green, and blue flesh fruits ($P < 0.05$ for all). Green fruits were also the least preferred by all species of wild-caught adults (Fig. 2B).

All juvenile individuals displayed a consistent preference for one or both of the common fruit colours (black and red) over ripe fruit of other colours. Overall, approximately 71% (22/31) of the total number of hand-raised individuals showed a preference for black fruit, 13% (4/31) preferred red fleshy fruit, and the remaining 16% (5/31) preferred both black and red fleshy fruit over fruit of other colours (Appendix S1-2).

There seemed to be a larger inter-individual variation in natural fruit colour preference among wild-caught adults than was observed for hand-raised juveniles. However, if red and black are lumped together as "common colours", then 25 of the 26 wild-caught birds (96%) showed a significant preference for one of the common fruit colours (including 4 individuals that preferred both a common colour and one other), and only one individual (4%) totally avoided the common colours of natural fruits, preferring to consume yellow fruit (Appendix S2-2).

Temporal variations in preference. Artificial fruit colour preference was stable over time for hand-raised juveniles and red was always the most preferred colour (Table 1). For natural fruits, black was the most stable and preferred colour by juvenile *P. aurigaster* during the entire experimental period. Hand-raised *P. jocosus* and *M. asiatica* showed a similar pattern in that they preferred black fruit on the first few days of the trial period, then expanded their preference to include both black and red fruit on subsequent days of the trial period (Table 1). In general, only the common colours were constantly preferred by all hand-raised birds for the entire trial period.

Artificial red fruit was also the most stable and preferred colour for wild-caught adults over time (Tables 2). The fleshy fruit colour preferences of wild-caught adults varied more substantially than those of juveniles of the same species. Wild-caught *P. jocosus* preferred red natural fruit on the first day of trials, both black and red fruit on days 2–4, black fruit on day 5, and again preferred black and red fruit on days 6–8. *P. melanicterus* showed a preference for red and yellow

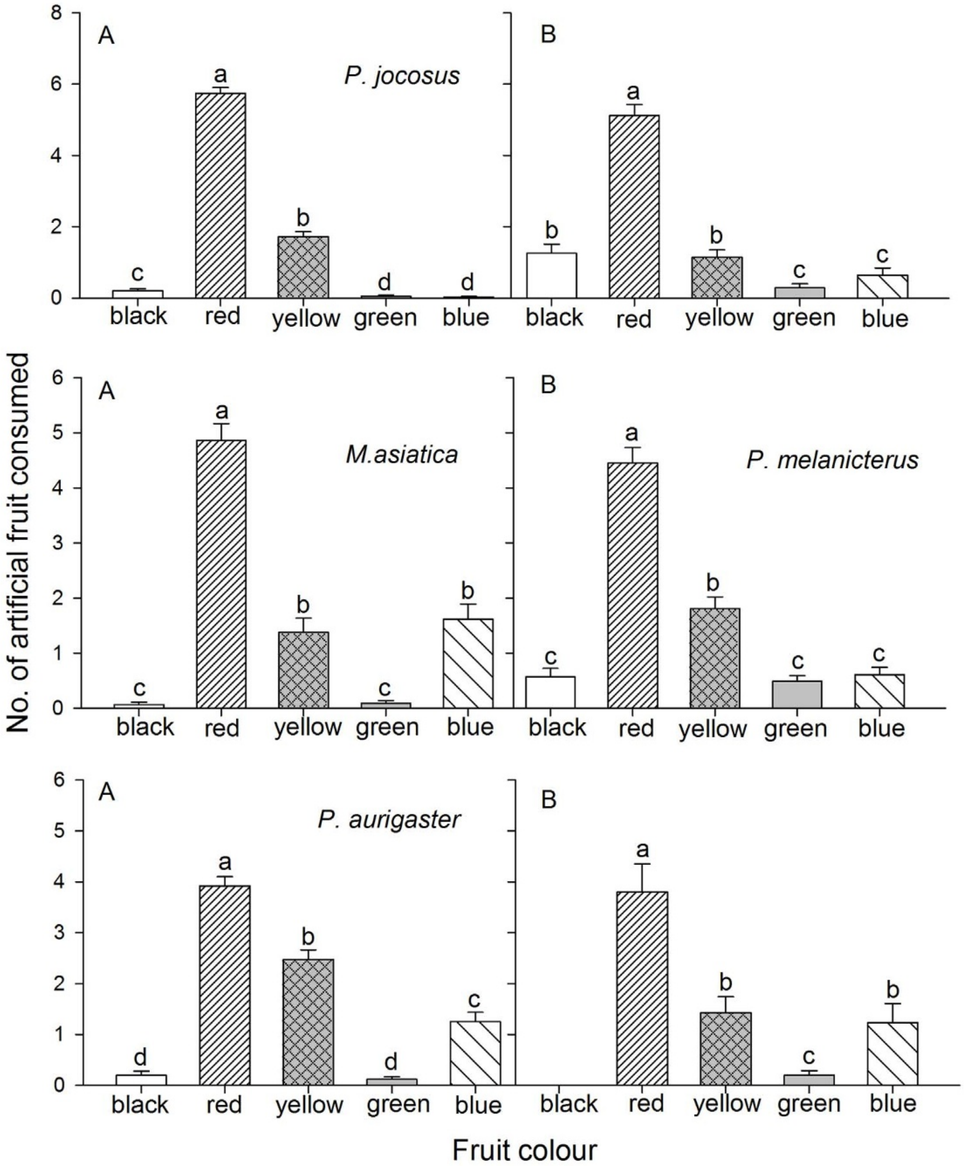

Figure 1 | Mean (±SE) number of artificial fruits consumed by hand-raised (A) and wild-caught bird species (B). Different letters within each panel indicate statistically significant differences ($p < 0.05$) between these five colours (ANOVA followed by multiple Bonferroni-corrected two sample T-tests).

fruit on the first day of trials, and then mainly focused on black and red fruit during the remainder of the trial period. During the first few days of the trial, wild-caught *P. aurigaster* and *M. asiatica* showed a preference not only for black and/or red fruit, but also for blue fruit. These birds then preferred only black and/or red fruits for the remainder of the trial period (Table 2). In general, a preference for common-coloured ripe fruit was the most stable and a consistent preference shown by all wild-caught birds over time (Table 2).

Discussion

Colour preferences of wild-caught experienced birds. The significance of fruit colour diversity in animal foraging decisions remains contentious, but if plants have adapted fruit colours that attract bird frugivores[6], frugivorous birds should exhibit a preference for the common colours, black and/or red fruits, over fruits of uncommon colours. We examined this assumption and found that all experienced wild *P. jocosus*, *P. melanicterus*, and *P. aurigaster* caught in Xishuangbanna, tropical Asia exhibited a strong and consistent preference for red artificially coloured fruits over others. This result is similar to that of some studies in which birds displayed a preference for red fruit[6,17,19], and consistent with our previous work[34], but contrasts with other studies in which birds displayed a preference for colours other than red or for no specific colour[15,24,25,35]. In the present study, the consistent preference of all wild-caught birds for red artificial fruit seems to support our hypothesis that birds prefer the most commonly encountered fruit colours, as blue, green and yellow are comparatively rare in the forest[20]. Surprisingly, red was preferred to black, even though black is actually more frequent in the study area (40% of fruit) than red (19%). However, the fact that no bird species in the experiment preferred artificially coloured black fruit is likely because the artificially coloured 'black' used in our experiment had a reflectance peak in the blue range that may have led birds to perceive it as blue[34]. All other artificial colours had reflectances which would lead birds to perceive them as the same colour humans perceive them as[34]. In these experiments more birds consumed red and yellow fruits than would be predicted by the frequency of natural fruits of those colours, but this was possibly caused by the lack of truly black artificial fruits in the experiment.

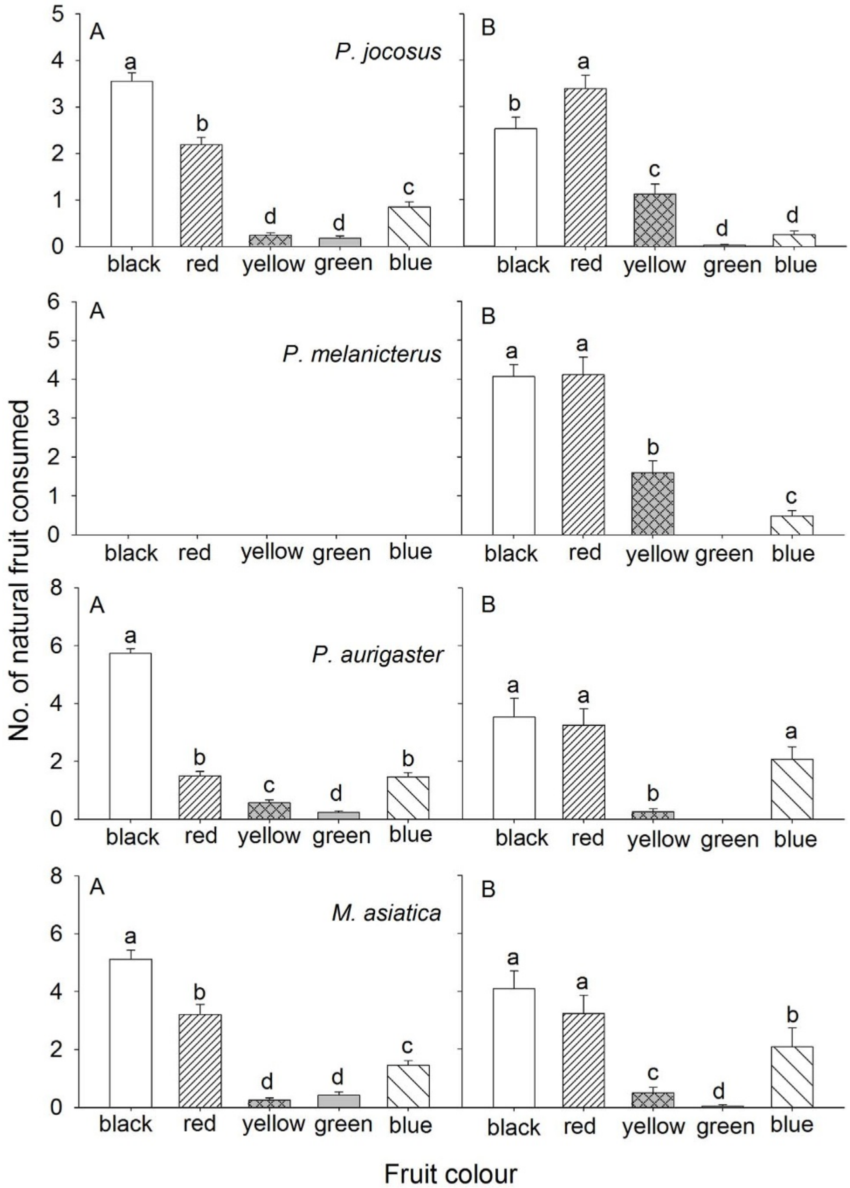

Figure 2 | Mean (±SE) number of fleshy fruits consumed by hand-raised (A) and wild-caught birds (B). Different letters within each panel indicate statistically significant differences ($p < 0.05$) between these five colours (ANOVA followed by multiple Bonferroni-corrected two sample T-tests).

Although black is one of the most common colours of fleshy fruit dispersed by birds, only a few studies of bird fruit colour preference have included black fruits in their experiments (e.g.[12,15,19,24,36]), and in very few of these studies have birds showed a preference for black fleshy fruits[36]. Furthermore, these previous studies on colour preference primarily used cultivated fruits or migrant bird species. In comparison to resident birds, migrants may have experienced more variable environments and learned to eat a broader range of differently coloured food items during migration[37]. Therefore, it is probably not reasonable to expect migrant birds to choose fruits based primarily on the frequency of fruit colours in the environment where they were caught. This might explain why the authors of many previous reports failed to find a positive relationship between the frequency of a fruit colour in the environment and the colour preference of frugivorous birds. In contrast, the natural experience of resident birds could be more reasonably expected to reflect the frequency of fruit colour in the local environment.

Using five common fleshy fruits of native plant species and four wild-caught resident bird species, our study showed a consistent and strong preference for both black and red fleshy fruits over ripe fruits of other colours. Although the different foraging experiences of the wild-caught experimental individuals would no doubt influence food choices[24,25,38], the wild caught resident birds overwhelmingly preferred black and red fleshy fruit. This is consistent with our previous studies[39]. However it should be noted that this study used different species of natural fruits than Duan and Quan[39], which used *Litsea glutinosa* for black, *Rauvolfia vomitoria* for red, *Duranta erecta* for yellow, and *Microcos nervosa* for green. The fact that the two studies showed similar results despite using different fruiting species suggests that that black and red are the preferred colours of fruit for birds in our study site. As indicated by previous studies, the reason birds prefer these colours might be because black and red are especially conspicuous against natural backgrounds compared to other fruit colours[12], as well as the frequency of these colours in nature in this region.

To posit birds as a selective force on fruit colour, we must also demonstrate a preference against ripe fruit of less common colours. All wild-caught birds in our study consistently avoided artificial and natural ripe green fruits. These results are consistent with most previous studies, in which green fruits were avoided by frugivorous

Table 1 | Stability of fruit preference by hand-raised juvenile birds during the continuous trial period (day)

		Artificial fruits								Natural fruits								
Species	Colour	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9
P. jocosus	black									✓	✓	✓	✓	✓	✓	✓	✓	✓
	red	✓	✓	✓	✓	✓	✓	✓	✓						✓	✓	✓	✓
	yellow																	
	green																	
	blue																	
P. aurigaster	black									✓	✓	✓	✓	✓	✓	✓	✓	✓
	red	✓	✓	✓	✓	✓	✓	✓	✓									
	yellow																	
	green																	
	blue																	
M. asiatica	black									✓	✓	✓	✓	✓	✓	✓	✓	✓
	red	✓	✓	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓	✓
	yellow																	
	green																	
	blue																	

✓: preferred at $p < 0.01$ level, based on ANOVA followed by multiple Bonferroni-corrected two sample T-tests.

birds[25,30], and our previous work[34,39]. Some studies attributed the avoidance of green to its relative inconspicuousness[6,12], while others showed green was conspicuous against background[12,19]. However, most studies agree that green might signal unripeness and birds might use green as a cue to recognize unripe fruits[6,19]. Although the reason for the avoidance of green fruits by birds is still an open question, if bird preferences affect fruit colour, the consistent and constant pattern of avoiding ripe green fruits shown by all wild-caught individuals might explain why ripe green is the least frequent fruit colour in our study site. As in some previous reports[1,3], we also observed that ripe blue and yellow fruits, which occur relatively infrequently in our study site, were only occasionally consumed by wild-caught individuals (Fig. 2, Table 2, Appendices S2-2).

Besides contrasts, some other selective pressures have also been reported to influence fruit colour. For example, anthocyanins may be important drivers of fruit colour evolution because of their protective properties for plants (e.g. they reduce the growth of fungi found in fruit tissue[21]). Further, birds also may seek out anthocyanins for their antoxidative properties, and birds could use colour as a reliable indicator of the anthocyanin contents of fleshy fruits[22]. In addition, phylogenetic effects are also reported to be the factors that influence fruit colour evolution in our study site, accounting for approximately 52% of the variance in fruit colour[20]. However, a recent article that investigated phylogenetic effects on fruit colour at both global and local levels found "little indication of phylogenetic conservatism[10]".

Colour preferences of hand-raised naïve birds. The naïve birds used in this choice trial had no prior experience with fruit colours. As hand-raised juveniles of all species showed a strong and consistent preference for red artificial fruits, we suggest that juveniles have an unlearned preference for red fruit. Hand-raised blackcaps (*Sylvia atricapilla*; Schmidt and Schaefer[25]), blackbirds (*Turdus merula*; Larrinaga[19]) and northwestern crows (*Corvus caurinus*; Willson and Comet[30]) also showed a similar tendency to prefer red foods. An innate preference for red might suggest that the commonness of red fruits helps juveniles to recognize palatable food resources[25], or at

Table 2 | Stability of fruit preference by wild-caught adult birds during the continuous trial period (day)

		Artificial fruits						Natural fruits							
Species	Colour	1	2	3	4	5	6	1	2	3	4	5	6	7	8
P. jocosus	black								✓	✓	✓	✓	✓	✓	✓
	red	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
	yellow														
	green														
	blue														
P. aurigaster	black							✓	✓		✓	✓	✓	✓	✓
	red	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
	yellow														
	green														
	blue							✓	✓	✓	✓				
P. melanicterus	black								✓	✓	✓	✓	✓	✓	
	red	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓
	yellow							✓							
	green														
	blue														
M. asiatica	black							✓	✓	✓	✓	✓	✓	✓	✓
	red									✓	✓		✓	✓	
	yellow														
	green														
	blue							✓	✓	✓					

✓: preferred at $p < 0.01$ level, based on ANOVA followed by multiple Bonferroni-corrected two sample T-tests.

least function as an important signal in food discovery[29]. However, in interpreting these results it should be remembered that the artificial black colour in this study may have been perceived as blue, as explained above.

Despite a consistent preference for artificial red food, all hand-raised juveniles from all three species showed a strong and consistent preference for black fleshy fruits to red and to fruits of other colours. The same pattern was seen when only analysing the first fruit an individual consumed (Appendix S3). This differs from the preferences shown by conspecific wild-caught birds, which preferred both black and red fleshy fruits over the fruits of other colours. In Xishuangbanna, the majority of ripe fruit are black (40%), and red are the next most common (19%). Therefore, the juveniles' unlearnt consistent preference for the black fleshy fruits of native species over the less common red fruit suggests that the frequency of natural fruit colour is better reflected by the innate preferences of these birds than by the preferences of wild-caught adults. Indeed, red fruits may be either ripe or unripe in nature, and therefore red flesh is not always a reliable signal of palatability. In contrast, the colour of natural black fruits always signals palatability[2,30], and thus it would be adaptive for juveniles to prefer black fruit over red. In addition, wild-caught birds have experienced a variety of differently coloured fruits in the wild. Because previous experience affects future choices[24,28,38], it is probably not adaptive for these wild-caught birds to choose food based only on colour frequency. Consequently, red (the second-most common colour) may operate more strongly as a cue for food detection in experienced birds than in inexperienced birds.

Two other factors, the contrast of fruit colour against a background, and the nutrition of fruits, could have influenced the decisions of birds. Conspicuousness against a background has been shown to be an important factor in birds' fruit preferences[11–13,22]. In our experiments it is not clear whether conspicuousness was a factor because all fruits were displayed against an artificial white background. In previous studies[34], we have looked at the effect of contrast on fruit colour preferences of artificial fruit and have found that it doesn't change birds' basic preferences for certain colours like red; nevertheless, the effect of contrast should be explored in further work on the colour preferences of frugivores for wild fruits in this region. As to nutrition, it is possible that the natural fruits had different nutritional values; however if this was the case, we would expect changes over time in the birds preferences, which in general were not found (please see section on temporal variation, below). Further, as mentioned above, the hand-raised birds' first fruits chosen were predominantly black, and then red.

Inexperienced birds of all three species showed a strong and consistent innate avoidance of both artificial and natural ripe green fruits. Although the avoidance of green may be easily overcome by experience[35], the significant and consistent pattern of avoidance of ripe green fruits by both experienced and inexperienced frugivores in this study implies the existence of constant selective pressures exerted by bird dispersers against some less-common fruit colours (e.g., green).

Inter-individual variations in colour preferences. To assess whether food preferences by bird dispersers exert a stable selection pressure on the frequency of fruit colour, the preference pattern should be consistent and not easily changed[6,16,30]. Although some studies have found a preference for certain colours, most of them have shown strong intra-specific variation[12,17,30]. These strong inter-individual variations imply that there is a lack of consistent and constant selective pressure exerted by bird dispersers on the frequency of fruit colour.

In our study, the inter-individual variations in colour preference were very weak (Appendices S1-1 and S2-1). Nearly all of the hand-raised and wild-caught birds preferred red artificial fruits (86% and 90%, respectively). The inter-individual variation in natural fruit colour preferences might appear to be relatively large among wild-caught individuals, as some individuals preferred black, some preferred red, and some preferred both. However, approximately 96% (25/26) of wild-caught birds significantly preferred one or both of the common-coloured (red or black) fruit (Appendix S2-2). It seems the differences in the experiences of wild-caught birds did not ultimately lead to strong inter-individual variation in colour preference for uncommon colours. Perhaps more significantly, all hand-raised birds (3 species, 31 individuals) showed a consistent preference for common-coloured fleshy fruits (Appendix S1-2). The consistent pattern exhibited by almost all individuals, whether wild-caught or hand-raised, implies the potential for directional selective pressures on natural fruit colour towards the common black and red fruit colouration observed in this area.

Temporal variation in colour preferences. Learning and experience can alter colour preference pattern of birds[25,38,40], but if birds change their preferences during a short period in a response to external stimuli, they could not exert a constant selective force to drive fruit colour evolution. Therefore, temporal variation in colour preference might have important evolutionary consequences for plant-animal interactions. Both wild-caught and hand-raised birds in our study showed a stable preference for red artificial fruits over a several-day period (Tables 1 and 2). The colour of natural ripe fruits favoured by wild-caught birds shifted somewhat between trials; during the first few trials, wild-caught birds preferred both common (red and/or black) and rare (blue) fruit, but as the trials continued, wild-caught birds preferred only the common black and red fleshy fruits. All hand-raised juveniles showed a stable preference for black natural fruits, although their preference for red fruits increased over subsequent trials until they preferred both black and red fruit (Table 2). It seems that the preference for natural black by hand-raised birds reflected an inherited preference at the beginning of experiment which was modified with ongoing positive experience with red fleshy fruits. The highly constant preference towards the most common black and red fruit over time shown by wild-caught and hand-raised birds also implies a stable selective pressure that is reflected in the commonness of black and red fruit.

A limitation of our study is that for each fruit colour we only presented one species of fruit (as mentioned above, another study of our group exclusively looking at experienced adults found similar results with a different set of species). This limitation could be a problem for the interpretation of our results if the fruit species had different nutritional contents, because nutritional contents could influence food selection[9,21]. Hence further studies testing nutritional contents of these plant species are needed to see whether these traits also influence fruit choice. For now, we think it is reasonable to suggest that fruit colour is the dominant force behind birds' preferences, because if fruit nutrition is very poor we would expect wild birds to avoid such fruits. In contrast, we observed wild birds eating these fruits frequently (Duan, personal observation). Further, if there had been large nutritional differences between the fruit species, we would expect to see changes over time in the birds' preferences in our study. For example, we might expect birds to focus on only just red or just black fruits by the end of the experiment if one of these fruits had the highest nutritional content, not a mixture of both. It is possible that red and black fruits both had higher nutritional content than other fruits; yet we point out that for juvenile birds the first fruit consumed tended was most often black, and next often red. Fruit size and shape are other important characteristics that may influence bird food selection[41,42]. However, the fruits used in our experiment were similar in size and in (roughly oval) shape, so we believe fruit colour explains most of the differential response to fruits in this study.

Conclusions. Our study, using resident hand-raised and wild-caught birds, and both artificial and naturally ripe fruit of native plants, allows us to conclude the following: (1) All experimental birds have a strong preference for the most common fruit colours, black and red. We must acknowledge, however, that these same colours are also the most contrasting colours against a constant background, and hence contrast, as well as colour per se, may play a role in our results. (2) Relative to adult wild-caught birds, the innate colour preference of hand-raised birds, with a strong preference for black natural fruit, better reflects the frequency of natural fruit colour in our study site. Although experience influenced the selection of natural fruit colours by wild-caught birds to a certain extent, the colours (black and red) preferred by them were still the most common colours of fleshy fruit. (3) The preferences for common-coloured fruit and avoidance of ripe fruit of an uncommon colour are relatively invariant at inter- and intra-specific levels, and are also stable over time. Although we used only four species, which is a small number considering the diversity of Asian frugivores, these species are representative of some avian families of frugivores (bulbuls and barbets) that are dominant in human-modified landscapes in which larger frugivores are often extirpated[43], and such landscapes are, unfortunately, increasingly common.

Methods

Ethic statements. This study was carried out in strict accordance with the Guideline for the Care and Use of Laboratory Animals of China. The protocol was approved by the Administrative Panel on the Ethics of Animal Experiments of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (Permit Number: XTBG2011-3).

Study area and species. The study was carried out at Xishuangbanna Tropical Botanical Garden (XTBG, centred at 21°55′N, 101°16′E) in central Xishuangbanna Dai Autonomous Prefecture, southwest China. This region has an especially high biodiversity, which includes 16% of the higher plant species and 36% of the wild bird species found in China[44]. The forests in this region can be grouped into four types: tropical rain forest, tropical seasonal moist forest, tropical montane evergreen broad-leaved forest, and tropical monsoon forest[45]. In this tropical area, 40% of the 412 ripe fleshy-fruited plant species have black fruit, while 19% are red, 13% are brown, 13% are yellow, 8% are bicolour, and a smaller number have green, white, or blue fruit[20].

We tested the fruit-colour preferences of four bird species: *Pycnonotus jocosus*, *P. melanicterus*, *P. aurigaster*, and *Megalaima asiatica*. Both wild-caught and hand-raised individuals were tested for each species except for *P. melanicterus*, for which only wild-caught birds were used. *P. jocosus*, *P. melanicterus*, and *P. aurigaster* are members of the bulbul family (Pycnonotidae), and have mean body lengths of 19 cm, 19 cm, and 21 cm, respectively[46]. *M. asiatica* is a member of the barbet family (Megalaimidae) and has a mean body length of 20 cm[47]. We selected these species because they are the most abundant bird species at our study site, and the use of common birds facilitates finding nests and the acquisition of juveniles. Further, these birds are representative of important families of Asian avian frugivores. For example, of the bulbuls, R. Corlett (1998) writes: "Altogether, the available evidence suggests that the bulbuls may be most important seed dispersal agents in anthropogenic open habitats in the Oriental Region". The barbets are "among the most highly frugivorous birds in the region, west of Wallace's Line"[31]. In Xishuangbanna, these birds consume large amounts of fruit from a wide variety of plant species, as shown by their stomach contents, which are mainly made up of the remains of different fruits[46,47].

Ten *Pycnonotus jocosus*, seven *P. melanicterus*, and four *Megalaima asiatica* were captured in January and February 2011, before the breeding season. Five *P. aurigaster* were captured in December 2011 in the non-breeding season. We planned to catch 7–10 individuals of each experimental bird species, but the number of *P. aurigaster* and *M. asiatica* was constrained as they are canopy dwellers and are therefore difficult to capture. Birds were kept individually in 30 × 30 × 40 cm cages. The maintenance diet of all birds consisted of apple, pear, banana, and mealworms (which was given in a yellowish mixture); water was available *ad libitum*.

Juvenile *P. jocosus* (12), *P. aurigaster* (10), and *M. asiatica* (9) were taken from nests in XTBG between April and June 2011, 4–6 days after hatching. Juveniles were hand-raised and fed with apple, pear, banana, and mealworms. All fruit was peeled prior to feeding in order to prevent juveniles from acquiring colour preferences based on fruit peels. Field video observation showed that prior to collection, juveniles were fed mainly caterpillars and insects (Li H., unpublished data). Therefore, the juveniles in our experiment had no experience with any natural fruit items before the start of the experiment. Juveniles and adults were held in different rooms to prevent feeding imitation of adults by juveniles. Juveniles were placed in individual cages (30 × 30 × 40 cm) at 5–7 weeks of age, when they were able to feed themselves.

Fruit type and colour. Both artificial and natural fruits were used for colour selection experiments. Artificial fruit used in our experiments was a mixture of apple, pear, banana, wheat, and corn flour (1 : 1 : 1 : 1 : 1). We dyed the artificial fruit black, red, yellow, green, or blue using 0.01% tasteless food colouring[34]. The artificial fruits were shaped spherically with a diameter of 5–7 mm, similar to the natural fruit size consumed by bulbuls and barbets at our study site. Because the artificially coloured fruits were totally new to both wild-caught and hand-raised birds, the experiences of wild-caught birds with fleshy fruits might influence artificial food selection to a lesser extent than natural food selection. We therefore expected that both experienced and naïve birds would have similar patterns of preference for artificial fruit.

We also used ripe fruits of native species to test colour preference. Because we assumed that wild-caught birds had experience with these common fleshy fruits, and previous experience influences food preference later in life, we expected the colour preferences of experienced (wild-caught) and inexperienced (hand-raised) birds to differ, and specifically, for the innate preferences of naïve birds to better reflect the natural frequency of differently coloured fruit. We used naturally ripe fruits from five common species: *Phaeanthus saccopetaloides* (black), *Psychotria fluviatilis* (red), *Debregeasia orientalis* (yellow), *Ficus langkokensis* (green), and *Ophiopogon japonicus* (blue). While we unfortunately do not have reflectance spectra data for these species, spectra from other species of trees in this forest have generally been consistent with the qualitative categories of Chen et al[20] (see Quan and Duan[39]), and have little reflectances in the ultraviolet, making it likely that human categories may match the perceptions of birds. These fruits have no secondary structure, matured simultaneously during the trial period, were similar in size and in (roughly oval) shape, and were normally consumed by the experimental bird species in the field (Duan observ.). All species have dark green leaves.

Fruit traits, such as size and shape, nutritional value, and conspicuousness[14,35,42,48,49] affect fruit-bird interactions. However, as birds are visually-guided foragers, they commonly use colour/colour contrast as important signals to find and assess fruits[12,22,50]. Moreover, Valido et al.[9] found that traits (e.g. morphology, nutrients, and colour) of bird-consumed fruits showed a higher integration with colour than fruits consumed by other frugivorous animals. As these recent studies also indicated fruit traits and colour covary in birds consumed fruits, we ignored the potential effects of other fleshy fruit traits on bird food preferences in this study (as for the possible influence of variation among fruits in their nutritional value and morphology, please see discussion).

Experimental design. Four experiments were conducted to test bird colour preference: adult × artificial fruits, adult × natural fruits, juvenile × artificial fruits, and juvenile × natural fruits.

Artificial fruits. Adult × artificial fruit food-choice experiments were conducted in March–April 2011 and February 2012 after the wild-caught adults had habituated to the experimental cages. Juvenile × artificial fruit experiments were conducted in late August 2011, after juveniles were able to eat independently. Each adult bird participated in six trials and each juvenile bird participated in eight trials. Trials were conducted on consecutive days from 09:00–11:00 or 13:00–15:00. Before each trial, maintenance food was removed from the experimental cage for 1–1.5 h, water remained available *ad libitum*. At the start of each trial, birds were exposed simultaneously to 40 artificial fruits of five colours (eight fruits/colour × five colours), and all fruits were placed randomly in a transparent Petri dish with white background. We allowed the bird to feed until approximately 8–10 fruits were eaten (roughly equal to the number corresponding to any individual colour of fruit) and then counted the number of unconsumed artificial fruits. Each individual was tested separately to avoid influence on colour selection from other birds.

Natural fruits. Following the same method as artificial fruits, we displayed 40 naturally ripe fruits (eight fruits/colour × five colours) simultaneously to each of the birds. Each adult bird participated in eight trials and each juvenile bird participated in nine trials. Trials were conducted on consecutive days. Adult × natural fruit trials were conducted in December 2011. Juvenile × natural fruit trials were conducted in January 2012, three months after the end of juvenile × artificial fruit trials to minimize any affect that the experience of juveniles in the artificial food choice experiment might have on their decisions regarding natural fruits.

Data analysis. As a response variable, we used the number of fruits consumed by an individual bird of each colour per trial. We used ANOVA to determine whether food preferences of bird species of different ages (hand-raised juveniles versus adults) differed between these five colours. We then conducted multiple Bonferroni-corrected two sample T-tests, to further examine the differences between each pair of colours.

We first examined colour preferences pooling all individuals of the same age group of the same species. Because during the continuous experimental period, a bird might learn that certain characteristics of fruits (e.g. nutrition, texture) were identical (for artificial fruits) or different (for natural fleshy fruits), and decrease or increase their degree of selection among the five colours, we examined whether colour preferences were stable over the 6–8 consecutive days of trials. Preferences of each species for each day were determined by ANOVA followed by multiple Bonferroni-corrected two sample T-tests, as above. Finally, we measured inter-individual variation among individuals of a species in an age class in colour preference.

1. Janson, C. H. Adaptation of Fruit Morphology to Dispersal Agents in a Neotropical Forest. *Science* **219**, 187–189 (1983).
2. Wheelwright, N. T. & Janson, C. H. Colors of Fruit Displays of Bird-Dispersed Plants in 2 Tropical Forests. *Am. Nat.* **126**, 777–799 (1985).

3. Willson, M. F., Irvine, A. K. & Walsh, N. G. Vertebrate Dispersal Syndromes in Some Australian and New-Zealand Plant-Communities, with Geographic Comparisons. *Biotropica* **21**, 133–147 (1989).
4. Schaefer, H. M. & Schaefer, V. [The evolution of visual fruit signals: concepts and constraints] *Seed dispersal: theory and its application in a changing world* [Dennis, A. J., Schupp, E. W., Green, R. & Westcott, D. W. (eds.)] [59–77] (CBA international, Wallingford, 2007).
5. Sourd, C. & Gautierhion, A. Fruit Selection by a Forest Guenon. *J. Anim. Ecol.* **55**, 235–244 (1986).
6. Willson, M. F. & Whelan, C. J. The Evolution of Fruit Color in Fleshy-Fruited Plants. *Am. Nat.* **136**, 790–809 (1990).
7. Lomascolo, S. B. & Schaefer, H. M. Signal convergence in fruits: a result of selection by frugivores. *J. Evolution. Biol.* **23**, 614–624 (2010).
8. Lomascolo, S. B., Levey, D. J., Kimball, R. T., Bolker, B. M. & Alborn, H. T. Dispersers shape fruit diversity in Ficus (Moraceae). *P. Natl. Acad. Sci. USA* **107**, 14668–14672 (2010).
9. Valido, A., Schaefer, H. M. & Jordano, P. Colour, design and reward: phenotypic integration of fleshy fruit displays. *J. Evolution. Biol.* **24**, 751–760 (2011).
10. Stournaras, K. E. *et al.* How colorful are fruits? Limited color diversity in fleshy fruits on local and global scales. *New. Phytol.* **198**, 617–629 (2013).
11. Cazetta, E., Galetti, M., Rezende, E. L. & Schaefer, H. M. On the reliability of visual communication in vertebrate-dispersed fruits. *J. Ecol.* **100**, 277–286 (2012).
12. Schmidt, V., Schaefer, H. M. & Winkler, H. Conspicuousness, not colour as foraging cue in plant-animal signalling. *Oikos* **106**, 551–557 (2004).
13. Schaefer, H. M., Levey, D. J., Schaefer, V. & Avery, M. L. The role of chromatic and achromatic signals for fruit detection by birds. *Behav. Ecol.* **17**, 784–789 (2006).
14. Burns, K. C., Cazetta, E., Galetti, M., Valido, A. & Schaefer, H. M. Geographic patterns in fruit colour diversity: do leaves constrain the colour of fleshy fruits? *Oecologia* **159**, 337–343 (2009).
15. Willson, M. F., Graff, D. A. & Whelan, C. J. Color Preferences of Frugivorous Birds in Relation to the Colors of Fleshy Fruits. *Condor* **92**, 545–555 (1990).
16. Willson, M. F. Fruit Choices by Captive American Robins. *Condor* **96**, 494–502 (1994).
17. Puckey, H. L., Lill, A. & ODowd, D. J. Fruit color choices of captive Silvereyes (Zosterops lateralis). *Condor* **98**, 780–790 (1996).
18. Borgia, G. & Keagy, J. An inverse relationship between decoration and food colour preferences in satin bowerbirds does not support the sensory drive hypothesis. *Anim. Behav.* **72**, 1125–1133 (2006).
19. Larrinaga, A. R. Inter-specific and intra-specific variability in fruit color preference in two species of Turdus. *Integr. Zool.* **6**, 244–258 (2011).
20. Chen, J., Fleming, T. H., Zhang, L., Wang, H. & Liu, Y. Patterns of fruit traits in a tropical rainforest in Xishuangbanna, SW China. *Acta. Oecol.* **26**, 157–164 (2004).
21. Schaefer, H. M. Why fruits go to the dark side. *Acta. Oecol.* **37**, 604–610 (2011).
22. Schaefer, H. M., McGraw, K. & Catoni, C. Birds use fruit colour as honest signal of dietary antioxidant rewards. *Funct. Ecol.* **22**, 303–310 (2008).
23. Roper, T. J. Responses of Domestic Chicks to Artificially Colored Insect Prey - Effects of Previous Experience and Background Color. *Anim. Behav.* **39**, 466–473 (1990).
24. Honkavaara, J., Siitari, H. & Viitala, J. Fruit colour preferences of redwings (Turdus iliacus): Experiments with hand-raised juveniles and wild-caught adults. *Ethology* **110**, 445–457 (2004).
25. Schmidt, V. & Schaefer, H. M. Unlearned preference for red may facilitate recognition of palatable food in young omnivorous birds. *Evol. Ecol. Res.* **6**, 919–925 (2004).
26. Allen, T. & Clarke, J. A. Social learning of food preferences by white-tailed ptarmigan chicks. *Anim. Behav.* **70**, 305–310 (2005).
27. Clarke, J. A. White-tailed ptarmigan food calls enhance chick diet choice: learning nutritional wisdom. *Anim. Behav.* **79**, 25–30 (2010).
28. Slagsvold, T. & Wiebe, K. L. Social learning in birds and its role in shaping a foraging niche. *Philos. T R. Soc. B.* **366**, 969–977 (2011).
29. Gamberale-Stille, G., Hall, K. S. S. & Tullberg, B. S. Signals of profitability. Food colour preferences in migrating juvenile blackcaps differ for fruits and insects. *Evol. Ecol.* **21**, 99–108 (2007).
30. Willson, M. F. & Comet, T. A. Food Choices by Northwestern Crows - Experiments with Captive, Free-Ranging and Hand-Raised Birds. *Condor* **95**, 596–615 (1993).
31. Corlett, R. T. Frugivory and seed dispersal by vertebrates in the Oriental (Indomalayan) Region. *Biol. Rev.* **73**, 413–448 (1998).
32. Ingle, N. R. Seed dispersal by wind, birds, and bats between Philippine montane rainforest and successional vegetation. *Oecologia* **134**, 251–261 (2003).
33. Muscarella, R. & Fleming, T. H. The role of frugivorous bats in tropical forest succession. *Biol. Rev.* **82**, 573–590 (2007).
34. Duan, Q. & Quan, R. C. The Effect of Color on Fruit Selection in Six Tropical Asian Birds. *Condor* **115**, 623–629 (2013).
35. Stanley, M. C., Smallwood, E. & Lill, A. The response of captive silvereyes (Zosterops lateralis) to the colour and size of fruit. *Aust. J. Zool.* **50**, 205–213 (2002).
36. Galetti, M., Alves-Costa, C. P. & Cazetta, E. Effects of forest fragmentation, anthropogenic edges and fruit colour on the consumption of ornithocoric fruits. *Biol. Conserv.* **111**, 269–273 (2003).
37. Levey, D. J. & Stiles, F. G. Evolutionary Precursors of Long-Distance Migration - Resource Availability and Movement Patterns in Neotropical Landbirds. *Am. Nat.* **140**, 447–476 (1992).
38. Siitari, H., Honkavaara, J. & Viitala, J. Ultraviolet reflection of berries attracts foraging birds. A laboratory study with redwings (Turdus iliacus) and bilberries (Vaccinium myrtillus). *P. Roy. Soc. B-Biol. Sci.* **266**, 2125–2129 (1999).
39. Duan, Q. & Quan, R.-C. Natural fruit colour selection by frugivorous birds in Xishuangbanna. *Zool. Res.* **33**, 427–432 (2012).
40. Svadova, K. *et al.* Role of different colours of aposematic insects in learning, memory and generalization of naive bird predators. *Anim. Behav.* **77**, 327–336 (2009).
41. Wheelwright, N. T. Fruit size, gape width, and the diets of fruit-eating birds. *Ecology* **66**, 808–818 (1985).
42. Sobral, M., Larrinaga, A. R. & Guitian, J. Fruit-Size Preferences in Wild and Naive Eurasian Blackbirds (Turdus Merula) Feeding on Oneseed Hawthorn (Crataegus Monogyna). *Auk* **127**, 532–539 (2010).
43. Sekercioglu, C. H. Bird functional diversity and ecosystem services in tropical forests, agroforests and agricultural areas. *J. Ornithol.* **153**, S153–S161 (2012).
44. Zhang, J. H. & Cao, M. Tropical Forest Vegetation of Xishuangbanna, Sw China and Its Secondary Changes, with Special Reference to Some Problems in Local Nature Conservation. *Biol. Conserv.* **73**, 229–238 (1995).
45. Zhu, H., Cao, M. & Hu, H. B. Geological history, flora, and vegetation of Xishuangbanna, southern Yunnan, China. *Biotropica* **38**, 310–317 (2006).
46. Yang, L. *The avifauna of Yunnan, China (II)* (Yunnan Science and Technology Press, Kunming, Yunnan, 2004).
47. Yang, L. *The avifauna of Yunnan, China (I)* (Yunnan Science and Technology Press, Kunming, Yunnan, 1994).
48. Burns, K. C. & Dalen, J. L. Foliage color contrasts and adaptive fruit color variation in a bird-dispersed plant community. *Oikos* **96**, 463–469 (2002).
49. Schaefer, H. M., Schaefer, V. & Vorobyev, M. Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *Am. Nat.* **169**, S159–S169 (2007).
50. Schaefer, H. M. & Schmidt, V. Detectability and content as opposing signal characteristics in fruits. *P. Roy. Soc. B-Biol. Sci.* **271**, S370–S373 (2004).

Acknowledgments
This study was supported by National Nature Science Foundation of China (grant no. 31370452, 31170406) and a key project of the Chinese Academy of Science (KSCX2-EW-Q-17). Chen Jin of XTBG provided valuable discussion. We thank our field assistant Li Meng for technical support with the trapping and maintenance of birds.

Author contributions
Q.D. and R.-C.Q. designed the study, analyzed the data. Q.D. performed the experiments. R.-C.Q. wrote the manuscript. E.G. offered valuable comment and also wrote the manuscript.

Additional information
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Duan, Q., Goodale, E. & Quan, R.-C. Bird fruit preferences match the frequency of fruit colours in tropical Asia. *Sci. Rep.* **4**, 5627; DOI:10.1038/srep05627 (2014).