

Differential passage time of mistletoe fruits through the gut of honeyeaters and flowerpeckers: effects on seedling establishment

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Abstract. McKey's (1975) hypothesis that avian dispersers with a specialized gut provide higher quality seed dispersal than unspecialized frugivores was tested using grey mistletoe (*Amyema quandang*) fruits, and captive mistletoebirds (*Dicaeum hirundinaceum*) and spiny-cheeked honeyeaters (*Acanthagenys refogularis*) in arid South Australia. Mistletoebirds have a specialized gut, unlike spiny-cheeked honeyeaters. The gut passage time of *A. quandang* fruits through mistletoebirds was 820 ± 29 s (mean \pm SE, $n = 188$), compared to 2434 ± 36 s ($n = 436$) for honeyeaters. The seeds defecated by both bird species were deployed on twigs of host trees. Despite the longer retention time of fruit in the gut of honeyeaters, the germination percentage of seeds defecated by mistletoebirds (85% of 485 seeds) and honeyeaters (81% of 485 seeds) did not differ significantly 1 week after deployment. However, after 5 months, a significantly greater proportion of seedlings had established from seeds passed by mistletoebirds (42.7%) than from seeds defecated by honeyeaters (31.1%). The data support the notion that the more gentle treatment of seeds in the gut of specialized dispersers translates into higher seedling establishment.

Key words: Bird dispersal – Coevolution – Gut passage time – Mistletoe – Seedling establishment

Coevolution between fleshy-fruited plants and avian dispersers has resulted in adaptive fruiting displays in plants and morphological, anatomical and physiological specialization of frugivorous birds (Herrera 1985; Moermond and Denslow 1985). The selective pressures driving the coevolution between bird-dispersed plants and their dispersers are evidently the provision of food for birds by plants, in return for the dispersal of the plants' seeds. Bird-dispersed fruits differ in size, nutritional quality, morphology and presentation, and frugivorous birds

select their diet from among an array of fruits on the basis of these characteristics (Moermond and Denslow 1985). Likewise, the seed dispersal service provided by different avian dispersers has several dimensions (Snow 1971; Howe and Primack 1975; McKey 1975) and may differ significantly in some situations. Bird species vary in the quantity of fruits removed from the immediate canopy of the parent plant, their spatial and temporal reliability as consistent patrons of fruit crops, the treatment of seeds in the gut of dispersers, the efficiency with which seeds are deposited in "safe" sites, and the distance to which seeds are dispersed away from the parent plant.

One of the dimensions of seed dispersal quality emphasized by McKey (1975) was the treatment of seeds in the avian gut. He speculated that specialized frugivores with little muscularized stomachs, short digestive tracts and rapid fruit or seed passage times ought to damage seeds less through mechanical grinding and chemical digestion than unspecialized birds. He pointed to the soft-seeded lauraceous fruits consumed by specialized cotingids and the oilbird, and to the soft-fruited Loranthaceae dispersed by specialized euphonias and dicaeids. Rick and Bowman (1961) demonstrated that the retention of Galapagos tomato seeds in tortoise guts can reduce their germination. Nevertheless, direct evidence that the more rapid treatment of seeds by specialized frugivores translates into higher rates of seedling establishment is lacking. This paper tests McKey's (1975) hypothesis by first establishing a difference between the passage times of grey mistletoe (*Amyema quandang*) fruits through the gut of mistletoebirds (*Dicaeum hirundinaceum*) and spiny-cheeked honeyeaters (*Acanthagenys rufogularis*). We then contrast the establishment of mistletoes resulting from the seeds defecated by both species.

A. quandang is a common hemiparasite of western myall (*Acacia papyrocarpa*) in inland South Australia and is dispersed by both mistletoebirds (9 g) and spiny-cheeked honeyeaters (44 g) (Reid 1989, 1990). The mistletoebird specializes on mistletoe fruits and has a remarkably short digestive tract and reduced gizzard

(Richardson and Wooller 1988). The entrance to the gizzard is narrower than the entrance to the duodenum, so mistletoe fruits pass from the proventriculus to the intestine, bypassing the gizzard. The spiny-cheeked honeyeater has a more generalized diet of nectar, fruits and insects. Its gastrointestinal tract is "unremarkable" (R. Wooller, personal communication) compared to that of the mistletoebird, and is similar structurally and proportionally to other honeyeaters (Wooller and Richardson 1988).

The specific objectives of this study are to: (1) compare the rate of ingestion of *A. quandang* fruits by mistletoebirds and spiny-cheeked honeyeaters, (2) quantify the passage time of mistletoe fruits through the gut of each species, and (3) compare germination of seeds defecated by mistletoebirds and honeyeaters and the resultant infection of western myall by the seedlings.

Methods

The work was conducted at the Middleback Field Centre for Arid Zone Studies (137° 24' E, 32° 57' S) in South Australia, in January and February 1991. Mistletoebirds and spiny-cheeked honeyeaters were mistnetted as required, and held in aviaries (0.6 × 0.6 × 0.6 m) in an open-air barn for several days. Mistletoebirds were maintained on a diet of *A. quandang* fruits and were hand-fed a daily supplement of a protein-carbohydrate mix (Farex Infant Formula). The honeyeaters were maintained on a solution of 4 parts Farex Infant Formula to 1 part sucrose. Water was provided ad libitum at all times. The weather was fine throughout, with daily minima and maxima varying between 15–24° C and 24–44° C, respectively, at Whyalla (21 km south-east of Middleback).

Gut passage time

The passage time of mistletoe fruits was determined by recording the time of ingestion of each mistletoe fruit and the time of defecation of each seed. Gut passage time (GPT) for each fruit was calculated as the difference between the times of ingestion and defecation, which assumed that seeds were defecated in the order that they were ingested. This hypothesis was tested using dyed fruit. Two mistletoebirds and two spiny-cheeked honeyeaters were offered 8, 9, 9 and 11 *A. quandang* fruits respectively, dyed with food colouring of four different colours. Each bird defecated the dyed seeds in the same order as they were ingested, supporting the hypothesis that fruits do not change order during passage through the gut of the two species.

The GPT of mistletoe fruits through four mistletoebirds (three males, one female) and six spiny-cheeked honeyeaters was measured in feeding sessions starting at three different times of day: 0730 (early morning), 1100 (late morning) and 1845 hours (late afternoon). Between one and three replicate feeding sessions were conducted with an individual at each time of day. Feeding sessions lasted 1 h for mistletoebirds and 2 h for spiny-cheeked honeyeaters. The longer time for honeyeaters ensured that an adequate number of seeds was defecated during the session. Prior to feeding sessions, experimental birds were deprived of their normal diet for 1 h. This served to heighten the appetite of honeyeaters for *A. quandang* fruit and allowed time for mistletoebirds to clear their gut of fruits. During feeding sessions, experimental birds were provided with branches of *A. quandang* bearing abundant ripe fruits. Birds were observed from behind a screen and the exact times of fruit ingestion and seed defecation were recorded until the end of the session.

GPT was calculated for each seed defecated during a feeding session. However, because birds finished the session with a variable number of fruits in their guts, the data for each feeding session were truncated by subtracting the longest GPT of any seed passed by the

species (2283 s for mistletoebirds, 5050 s for honeyeaters) from the end of the feeding session. The GPTs of fruits ingested after that time were ignored. The truncated data contained 188 GPT values from 27 feeding sessions for mistletoebirds and 436 values from 30 feeding sessions for spiny-cheeked honeyeaters. A mean GPT was then calculated for each feeding session.

Two analyses of variance were performed in S (Becker et al. 1988). The S language is a programming environment for data analysis and graphics. It contains a full suite of operators for univariate and multivariate statistical analysis and data display, as well as a wide range of flexible facilities for both on screen and hardcopy graphical output. The first tested for differences between species in the number of fruits consumed in the first hour of feeding sessions (fruit consumption). The second tested for differences between species in mean GPT per feeding session. Log passage times were used to stabilize the error variance. Both analyses had (1) birds and (2) feeding sessions within birds as two error strata, and allowed for the possible covariate effect of daily temperature (mean of the daily maximum and minimum at Whyalla). Since individual birds were nested within species, the second error stratum contained no information on species differences, but provided information on variation with time of day and species by time of day interactions. Fruit consumption was also used as a natural covariate when testing for differences between species in GPT.

Germination and establishment of mistletoe seeds

A total of 454 and 457 *A. quandang* seeds defecated by captive mistletoebirds and honeyeaters respectively, were deployed on live western myall twigs, < 15 mm in diameter, between 31 January and 10 February 1991. Seeds were placed on 91 separate branches of 40 western myalls, in an alternating sequence of mistletoebird and honeyeater-defecated seeds. The position of individual seeds was marked with a paint spot. A further 31 and 28 seeds defecated by mistletoebirds and honeyeaters respectively, were placed on dead branches. Germination, defined as growth of the hypocotyl > 1 mm beyond the endocarp and endosperm, was recorded 7 days after deployment. Five months later, between 6 and 9 July, the fate of all seeds and seedlings was determined. The seedlings deployed on dead branches had exhausted their endosperm reserves and were dead, indicating that surviving seedlings on live branches had infected the host. Established seedlings had green hypocotyls that had formed a holdfast against the host branch.

Results

Ingestion and defecation

Analysis of covariance of the number of fruits ingested in the first hour of feeding sessions (Table 1) showed that between birds, neither species, temperature nor their interaction were significant. Within birds, temperature had a significant effect ($P < 0.05$) on fruit consumption, and time of day bordered on significance ($P = 0.0603$). Figure 1 shows that fruit consumption tended to decline on warmer days (Fig. 1a) and to decline across the day from early morning to late afternoon (Fig. 1b). These effects were more marked in honeyeaters than mistletoebirds, but the differences between species were not significant.

Mistletoebirds and spiny-cheeked honeyeaters differed greatly in the number of fruits maintained in the gut (Fig. 2). Mistletoebirds rapidly accumulated a mean of 3 fruits in the gut in the first 5 min, and maintained this number throughout a feeding session. By contrast, honeyeaters took 30 min to accumulate a mean gut

Table 1. Analysis of variance of the number of *Amyema quandang* fruits ingested by each species in the first hour of feeding sessions. Individual birds are nested under species

Factor	df	Mean-square	F	P
<i>I. Error: between birds</i>				
Temperature	1	18.0	0.121	0.7397
Species	1	358.8	2.418	0.1709
Temperature by Species	1	76.5	0.516	0.4997
Residuals	6	148.4		
<i>II. Error: within birds</i>				
Temperature	1	196.4	6.195	0.0170
Time of day	2	95.5	3.011	0.0603
Temperature by Time of day	1	81.7	2.576	0.1162
Species by Time of day	2	76.5	2.412	0.1023
Residuals	41	31.7		

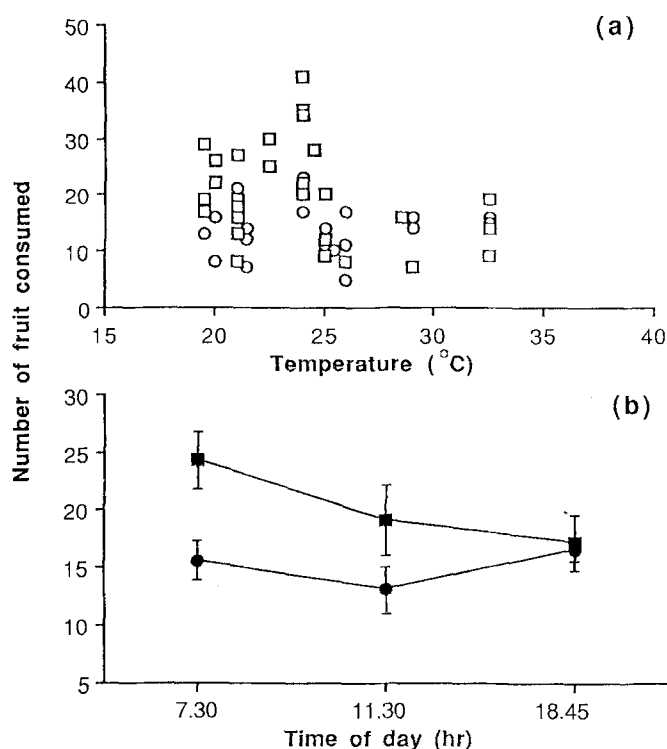


Fig. 1a, b. The effect of **a** daily temperature and **b** time of day on the number of *Amyema quandang* fruit consumed in the first hour of feeding sessions by spiny-cheeked honeyeaters (□, ■) and mistletoebirds (○, ●). Data in **b** are the mean number of fruits per feeding session \pm 1 SEM

capacity of 10–12 fruits. The number of seeds per defecation averaged 1.5 ± 0.05 (mean \pm SE, range 1–4, $n = 224$) for mistletoebirds and 3.8 ± 0.18 (range 1–16, $n = 192$) for honeyeaters.

Gut passage time

Mean GPT of mistletoe seeds passed by mistletoebirds and honeyeaters, pooled over all individuals and feeding

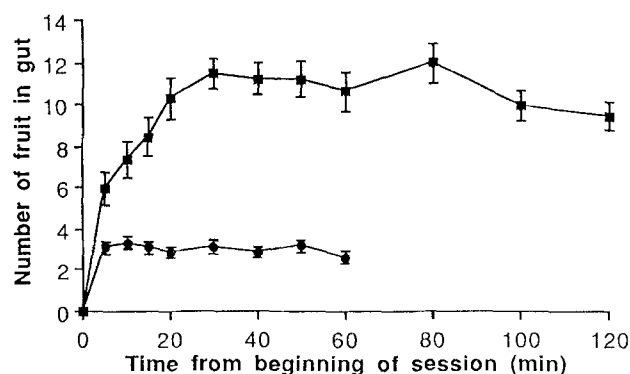


Fig. 2. Variation in the number of *A. quandang* fruits in the gut of spiny-cheeked honeyeaters (■) and mistletoebirds (●) through time. Data are the mean \pm 1 SEM at arbitrarily selected intervals from 30 honeyeater and 27 mistletoebird feeding sessions

Table 2. Analysis of variance of mean gut passage time of *Amyema quandang* fruits per feeding session. Individual birds are nested under species

Factor	df	Mean-square	F	P
<i>I. Error: between birds</i>				
No. of fruit	1	4.88	57.3	0.0001
Species	1	13.41	157.6	0.0000
Residuals	7	0.09		
<i>II. Error: within birds</i>				
No. of fruit	1	0.6336	8.276	0.0063
Time of day	2	0.2845	3.715	0.0327
Species by Time of day	2	0.2788	3.641	0.0348
Residuals	42	0.0766		

sessions, was 820 ± 29 s ($n = 188$ seeds) and 2434 ± 36 s ($n = 436$ seeds), respectively. The minimum and maximum GPT was 186 s and 2283 s for mistletoebirds and 949 s and 5050 s for honeyeaters, respectively. Analysis of variance of mean GPT per feeding session, between birds, revealed highly significant ($P < 0.001$) effects of both species and number of fruit (Table 2). Within birds, number of fruit was significant ($P < 0.01$) as was the interaction between species and time of day ($P < 0.05$). Mistletoebirds had a lower GPT than spiny-cheeked honeyeaters at all times of day (Fig. 3b). Mean GPT increased from early to late morning, particularly in honeyeaters. GPT in mistletoebirds declined by the late afternoon, whereas GPT in honeyeaters remained high. Increased fruit consumption was associated with a reduction in GPT, particularly in honeyeaters (Fig. 3a).

Variation in GPT within feeding sessions was also examined for each species. Pooling data across all feeding sessions and individuals, GPT was not correlated with the time of ingestion of each fruit from the beginning of the feeding session in mistletoebirds ($r = -0.01$, $n = 188$, NS), but was positively correlated with time of ingestion in honeyeaters ($r = 0.11$, $n = 436$, $P < 0.05$).

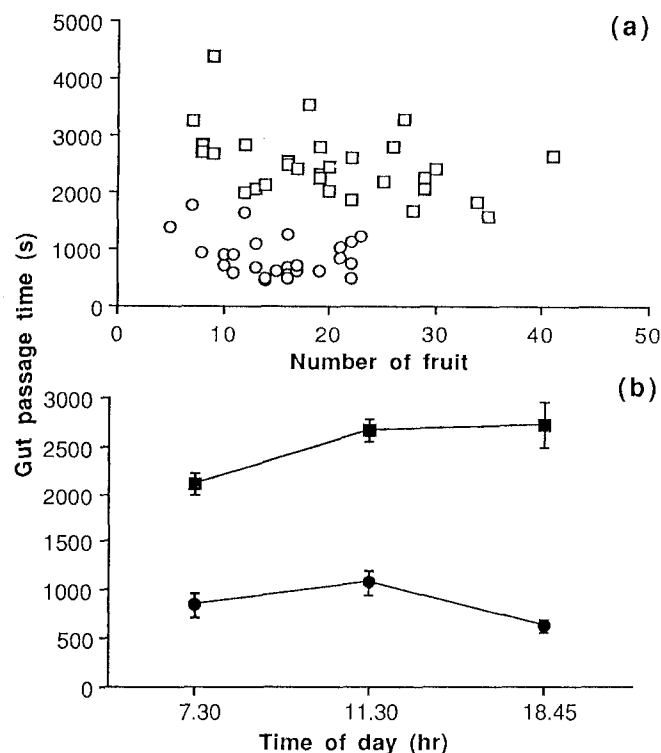


Fig. 3a, b. The effect of a fruit consumption and b time of day on the passage time of *A. quandang* fruits through spiny-cheeked honeyeaters (□, ■) and mistletoebirds (○, ●). Data in b are the mean gut passage time per feeding session \pm 1 SEM

Table 3. Fate of mistletoe seeds deployed on living western myall branches, after 5 months. Seeds were defecated by mistletoebirds and spiny-cheeked honeyeaters

Fate	No. of seeds (%)	
	Mistletoebird	Spiny-cheeked Honeyeater
Unknown	26 (5.7)	18 (3.9)
Predated	16 (3.5)	15 (3.3)
Dead	236 (52.0)	292 (63.9)
Established	176 (38.8)	132 (28.9)
Total	454 (100.0)	457 (100.0)

Germination and establishment of seeds

Many *A. quandang* seeds defecated by honeyeaters appeared to have lost more mesocarp (fruit pulp and viscid tissue) than seeds passed by mistletoebirds. Nevertheless, germination of seeds defecated by mistletoebirds and honeyeaters did not differ significantly 1 week after their deployment on western myall twigs (85% and 81% of 485 seeds, respectively, $X^2 = 1.64$, NS). Five months later, 7–9% of the seeds defecated by each species were missing or had been eaten by insects (Table 3). After discounting their loss, 42.7% of seeds defecated by mistletoebirds had led to successful seedling establishment, compared to 31.1% of seeds defecated by honeyeaters ($X^2 = 12.06$, $P < 0.001$).

Discussion

GPT and fruit consumption

The passage time of fruits through mistletoebirds was, on average, three times more rapid than through honeyeaters. The minimum, mean and maximum GPT of *A. quandang* fruits through mistletoebirds, of about 3, 14 and 38 min respectively, compare favourably with GPTs of 3–22 min reported for Tickell's and Javan fire-breasted flowerpeckers (*Dicaeum erythrorhynchos* and *D. sanguinolentum*) feeding on fruits of several mistletoe species (Ryan 1899; Ali 1931; Docters van Leeuwen 1954). The minimum, average and maximum GPT of mistletoe fruits through spiny-cheeked honeyeaters, of about 16, 41 and 84 min respectively, is consistent with values of 30–80 min reported by Keast (1958) for silvereyes (*Zosterops lateralis*; 10 g) feeding on *A. miquelii* and *A. gaudichaudii* fruits. Silvereyes have an unspecialized gastrointestinal tract (Richardson and Wooller 1986) compared to dicacids. The large differences in GPT between the mistletoebird and spiny-cheeked honeyeater presumably result both from the passage of fruit through the latter's gizzard and a longer alimentary tract.

The dynamics of fruit passage through the gut of the two species exhibited some similarities. Contrary to expectation, the larger honeyeaters did not consume significantly more fruit than mistletoebirds during the first hour of feeding, although this may have been due to the small number of individuals used in experiments. In both species, mean GPT tended to be smaller when larger numbers of fruit were consumed. This is presumably an adaptive involuntary response for increasing digestive efficiency. In frugivorous birds, ingestion rate is limited by digestion rate, so GPT tends to be rapid (Levey 1991). The cost of rapid GPT is low digestive efficiency, so birds are likely to obtain relatively greater nutritive benefit per food item if the GPT of small meals can be increased.

Honeyeaters took 30 min to fill their gut with 10–12 fruits (Fig. 1), whereas mistletoebirds achieved gut capacity (3 fruits) within 5 min of the start of feeding. Since abundant fruits were provided, honeyeater feeding rate appears to have been constrained by the rate at which mistletoe fruits pass through the gut. The rate at which fruits enter or leave the gizzard may restrict the spiny-cheeked honeyeater's ability to fill its gut more quickly.

In mistletoebirds, GPT did not vary in a consistent fashion through feeding sessions. However, with honeyeaters, GPT increased significantly as the feeding session progressed. Due to their specialized gut, mistletoebirds can rapidly defecate strings of 1–9 *A. quandang* seeds, whereas spiny-cheeked honeyeaters experience considerable difficulty in defecating large numbers of seeds (Reid 1991). The cumulative effect of many viscous mistletoe fruits in the unspecialized gut of honeyeaters evidently retards the passage of later fruits.

Diurnal variation in GPT was related to each species' metabolic requirements. After the overnight fast, both species had to re-establish energy reserves, resulting in rapid GPT early in the morning. GPT in both species

increased across the morning as energy reserves were replenished. By late afternoon, mistletoebirds were again rapidly processing mistletoe fruit, presumably to build up sufficient energy for the overnight fast, whereas honeyeaters maintained elevated GPTs through the late afternoon. Due to their larger size, honeyeaters are probably able to store energy for the overnight fast over a longer daytime period than mistletoebirds. The decline in fruit consumption in warmer weather probably has an energetic explanation as well. Inactive (captive) birds require less energy for thermoregulation and therefore less food, as ambient temperature approaches body temperature.

Captive spiny-cheeked honeyeaters and mistletoe-birds defecated an average of 3.8 and 1.5 mistletoe seeds per stool, respectively. These averages are approximately 30% and 50%, respectively, of the number of mistletoe seeds defecated per stool by the two species in the field (Reid 1989), and only 30% and 50%, respectively, of the average number of fruit held in the gut at full capacity by captive individuals. Since captive birds were unable to make extensive flights, the need to conserve energy and rapidly evacuate the gastrointestinal tract after feeding was reduced, and may have resulted in stools containing smaller numbers of seeds. If so, the GPT of fruits in captive birds may have been higher than passage times of birds in the field.

GPT is one of a number of factors that affects the dispersal distance of *A. quandang* seeds by mistletoebirds and spiny-cheeked honeyeaters. The shorter GPT of mistletoebirds presumably translates into a more restricted seed shadow around fruiting mistletoe than in the case of honeyeaters (cf. Reid 1989). The extent to which this is advantageous to mistletoe depends on the degree of adaptation of the mistletoe to the local population of hosts. Our studies of the infectivity of *A. quandang* show that highest establishment rates occur on hosts growing in the immediate vicinity of maternal mistletoe (Reid and Yan, in preparation). Thus, a tight seed shadow associated with rapid GPT is not necessarily disadvantageous, at least in terms of maintaining mistletoe populations over one to several generations.

Germination and establishment

Slightly more of the seeds defecated by mistletoebirds had germinated 1 week after deployment than those from honeyeaters, but the difference was not significant. Similar results were obtained by Reid (1989). Five months after deployment of the seeds on western myall branches, however, a significantly greater percentage of seedlings had established from mistletoebird-defecated seeds than from seeds passed by honeyeaters. The more rapid and presumably more gentle passage of *A. quandang* fruits in the mistletoebird gut translated into a higher rate of seedling establishment. The longer retention of fruits in the honeyeater gut appeared to result in the greater removal of protective viscin (mesocarp), perhaps exposing seedlings in the free-living phase to desiccation or pathogenic attack. Alternatively, mechanical grinding in

the honeyeater gizzard and the more prolonged exposure to digestive fluids may have directly damaged the endosperm and embryo of the seeds.

Conclusions

Reid (1991) suggested that guilds of small birds that specialize on a diet of mistletoe fruit may have coevolved with mistletoe, and that the primary selective pressure was the tendency for small birds to disperse seeds to the small branches which are most suitable for infection. The specialized digestive anatomy of dicaeids probably evolved to circumvent the difficulty of passing large numbers of viscous seeds. Our experimental results support McKey's (1975) notion that once small dispersers with specialized guts had evolved, the high quality dispersal service offered by these birds could have been an important secondary selective pressure leading to their coevolution with mistletoe.

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