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Coloration and pteridine pattern in a new, *yolk body* mutant of *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae)

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Abstract. A *yolk body* (*yb*) trait is the sixth body-colour mutation, discovered in *P. apterus*, affecting the pteridine biosynthesis. It causes a yolk-like coloration of the mutant body, except the eyes which remain red-pigmented, similarly as in wild-type bugs. While the *yb* homozygous nymphs and very young imagoes are characterized by yolk-pigmentation of epidermal cells, the body, particularly the forewings of older homozygous mutant adults, turn to yolk-orange colour and then become brick-red. The *yb* trait exhibits complete penetrance and uniform expression in both sexes. Eight different pteridines have been identified in the *yb* mutant nymphs and imagoes with use of the paper and thin-layer chromatography. Developmental aspects of the changes in pteridine content found in both studied traits are discussed.

INTRODUCTION

Generally, it is assumed that insects can synthesize a number of pteridines (Ziegler & Harmsen, 1969), which may be deposited not only in wings and other epidermal structures, but also in various internal organs. Studies on pteridines are important for several physiological reasons: they are required as co-factors of many enzymes (Ziegler & Harmsen, 1969), they play an essential role in electron transport within the cells (Rembold, 1975), and, with the purines, they serve as a storage form of excretory products (Harmsen, 1966). According to Forrest & Smith (1975), pteridines may interact even at the level of the genome. Recently, the general importance of pteridines has been accentuated in human medicine (Blair et al., 1989). Considering all these facts, more detailed studies on pteridine biosynthesis and their function in living systems appear to be rewarding.

Our knowledge about the nature and significance of insect pteridines has been based on studies in endopterygote insects (Purrmann, 1945; Albert, 1952; Vuillaume, 1969; Fuzeau-Braesch, 1972, 1985; Phillips & Forrest, 1980; Kayser, 1985; Pfleiderer, 1992). However, many of the fundamental chemical relationships underlying pteridine biosynthesis and its genetic control have been elucidated with the use of pigments mutants. Early works in this area were performed on the body-colour mutants of *Ephestia* (Becker, 1938; Kühn, 1941; Caspari, 1949) and *Drosophila* (Beadle & Ephrussi, 1936) species. Since then, pteridine differences have been studied in the eye-colour mutants of *D. melanogaster* Meigen (e.g., Hadorn & Mitchell, 1951; Ferré et al., 1986). The body-colour mutants of the silkworm, *Bombyx mori* L., have been very useful in this research (Tsusue & Akino, 1965; Tsusue et al., 1990; Iino et al., 1992).

However, there is little information upon the genetic aspects of pteridine biosynthesis and pigment distributions within heteròpteran species. Only a few species, the large milkweed bug, *Oncopeltus fasciatus* (Dallas) (Smissman & Orme, 1969; Lawrence, 1970); *Dysdercus* species (Hollweg, 1972; Melber & Schmidt, 1992, 1994) and the firebug, *Pyrrhocoris apterus* (L.) (Němec & Socha, 1988; Socha & Němec, 1992) have been investigated in this respect.

The simplicity of laboratory breeding and the occurrence of several body-colour mutations of *P. apterus*, provided a unique opportunity to initiate a complex study on the genetic relationships between pteridine patterns and biochemical alterations in their metabolic pathway in this heteropteran species (Socha, 1993). The *white* (*wh*) trait was the first pteridine mutation discovered in *P. apterus* (Rizki & Sláma, 1968). This trait, characterized by inhibition of red pigment synthesis, was shown to be inherited as a single autosomal recessive. A *yellow* (*yw*) trait, the second pteridine mutation with the same mode of an inheritance as the first, caused a yellow-body coloration of *P. apterus* (Socha, 1984). To date, three other body-colour mutations affecting pteridine biosynthesis in this species have been described: the *mosaic* (*mo*) mutant, inherited as an unstable sex-linked recessive (Socha, 1987) and two autosomal dominant mutants, *Pale* (*Pa*) and *Apricot* (*Ap*) (Socha, 1988a,b).

All the five body-colour mutations of *P. apterus* have been analysed by using paper and thin-layer chromatography (TLC) (Socha & Němec, 1992). Many qualitative and semiquantitative pteridine differences among these mutants were revealed, which provided the basis for further characterization of the role of individual mutant genes in the biosyntheis of pteridine pigments in heteropterans. However, more pteridine mutations of this bug need to be investigated in order to extend our knowledge in the genetic regulation of the pteridine metabolic pathway.

Therefore, the present paper includes a morphological description of a new, *yolk body* (*yb*) mutation of *P. apterus* and its characterization by the pteridine patterns, revealed by TLC and paper chromatography.

MATERIAL AND METHODS

Experimental animals

In 1992, one hundred specimens of *P. apterus* were collected at location in the vicinity of Ma'agal Michael (MM) in Israel. The adults taken from the natural population were transferred to constant laboratory conditions. The MM stock culture was reared on linden seeds and water ad libitum at 26 ± 1°C, and under long-day (16L : 8D) photoperiod, allowing continuous reproduction. Five specimens of the new, *yb* mutation, found among F_2 progeny of this MM stock culture, were isolated to establish a breeding mutant strain. The breeding of the *yb* mutant strain was performed under the same conditions as in the bugs of the MM stock culture.

To determine the pteridine patterns, the nymphs from the MM stock culture (i.e., the wild-type) and of the *yb* mutant strain were reared in 0.5 l glass jars with water and linden seeds which were replenished twice a week. Freshly ecdysed nymphs of the 5th (last) instar and imagoes of both sexes were separated daily from the MM stock and mutant cultures and transferred into small Petri dishes in groups of 3–5 specimens. They were supplied with food and drinking water.

The nymphs of the 5th instar and the one week old imagoes of the *yb* and wild-type phenotypes were photographed.

Pteridine standards

L-biopterin, L-neopterin, D-neopterin, D-monapterin, L-sepiapterin, leucopterin, pterin, isoxanthopterin and lumazine were purchased from Schircks Laboratories (Jona, Switzerland) and xanthopterin was obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Standards were developed in darkness, in the same way as other samples, and detected under high intensity UV light (254 nm), according to Pfleiderer's data (1992). Since some of the standards produced several fluorescence spots after chromatographical development (probably isomers of the basic compound), identification of these pteridines was rather difficult. Nevertheless, the possession of some standards (e.g., neopterin) in both D and L forms made their identification possible. The only complication was with violapterin I, the dark-violet fluorescing pteridine revealed by paper chromatography (R_f 0.39) and described in Socha & Němec (1992), because no corresponding standard was available.

Analytical methods

SAMPLE PREPARATION. 4-days-old nymphs of 5th instar, 2- and 20-days-old imagoes of the wild-type and the *yb* mutation were used for the analyses of pteridines. Pteridines were analysed separately in bodies and eyes in males and females. The samples contained eyes or eyeless bodies from either 15 nymphs or 10 imagoes. Insect samples were pulverized in liquid nitrogen and then lyophilised; each vial was then filled with nitrogen, closed and the stopper was sealed with paraffin. They were stored in the refrigerator (–20°C) under nitrogen atmosphere until use. Then the samples were twice extracted by a mixture of methanol : acetic acid : water (4 : 1 : 5), the supernatants were evaporated and the samples were used for TLC and paper chromatography.

TLC ANALYSIS. Silica-gel TLC plates were washed with 70% ethanol and dried. Before use, they were activated by exposing them to 110°C for 2 h, and then saved in desiccator. The samples were developed ascendently in n-butanol : 5N acetic acid (4 : 1) twice. Pteridines were determined according to their R_f values and the colour of fluorescence under the light intensity UV light 254 nm (Pfleiderer, 1992) in comparison with standards.

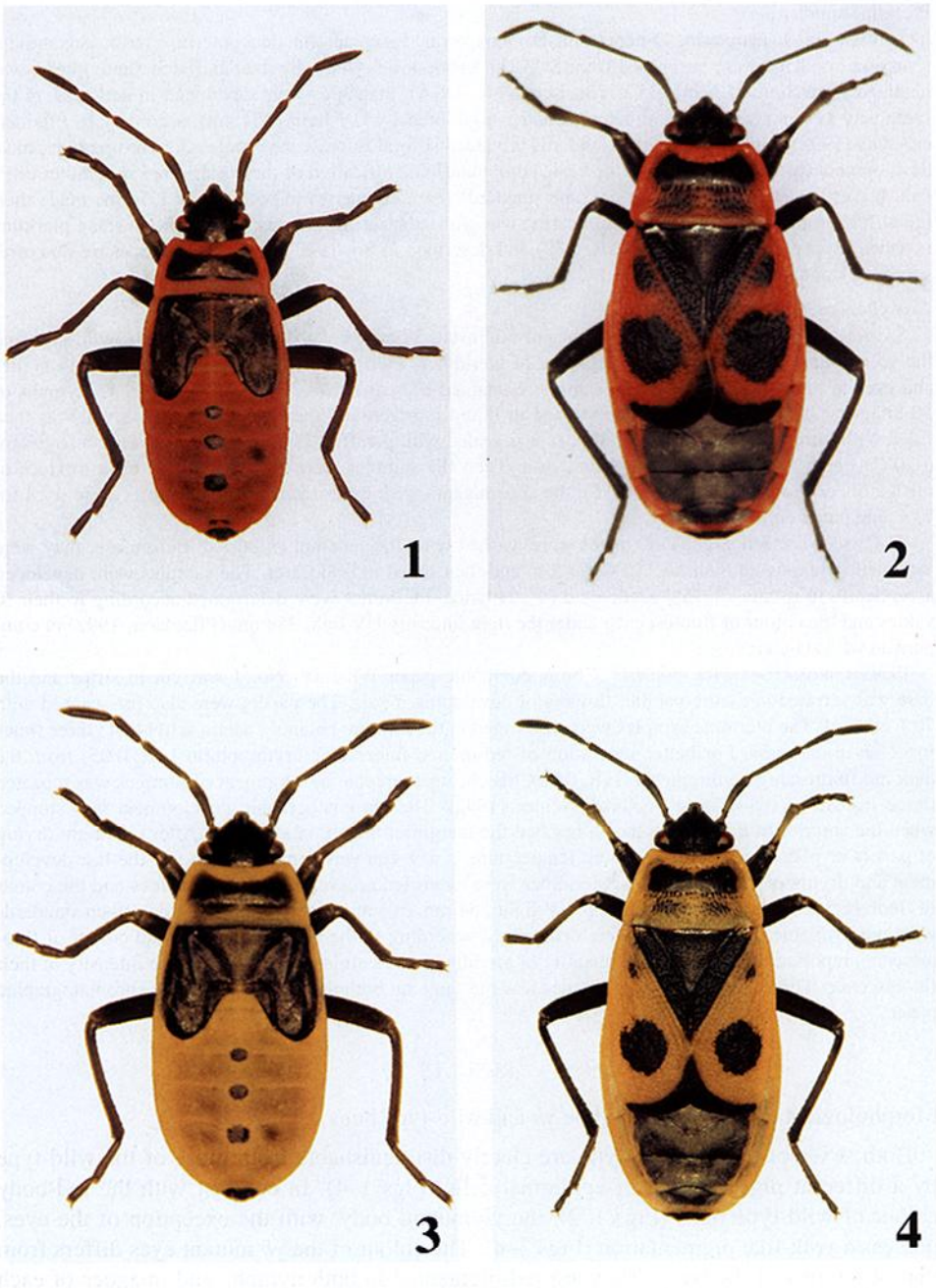
PAPER CHROMATOGRAPHY ANALYSIS. Chromatographic paper Whatman No. 1 was cut in strips and the base was serrated to assure parallel flowing of developing media. The papers were also pre-washed with 70% ethanol. The pteridine samples were developed in the mixture butanol : acetic acid (4 : 1) three times for 24 h in darkness. For better separation of red-orange fluorescing erythropterin I (R_f 0.05) from the dark red fluorescing erythropterin II (R_f 0.02), the chromatographic development of samples was repeated thrice instead of twice as in Socha & Němec (1992). The chromatographic development was stopped when the anterior of developing media reached the margin of the chromatograms. After overnight drying of papers or plates in darkness at room temperature, a new run was commenced. After the last development and drying of chromatograms, pteridines were identified according to their R_f values and the colour of fluorescence under the light intensity UV light 254 nm, in comparison with standards. When standards were not available, the pteridines were determined according to the relevant data (R_f and colour of fluorescence) reported in literature. The quantity of pteridines was estimated according to the intensity of their fluorescence. The fluorescence of pteridines was the same on both the silica-gel and the chromatographic paper.

RESULTS

Morphological characteristics of the *yb* and wild-type bugs

Both sexes of the *yb* phenotype are clearly distinguishable from those of the wild-type by a different pigmentation of epidermal cells (Figs 1–4). In contrast with the red-body colour of wild-type bugs (Figs 1–2), the *yb* mutant body, with the exception of the eyes, possess a yolk-like pigmentation (Figs 3–4). The colour of the *yb* mutant eyes differs from that of the rest of the body. They are red-pigmented in both nymphs and imagoes of each sex, as in the wild-type bugs.

The yolk-like coloration of the body is visible in the mutant nymphs until the 4th instar; nymphs of the 5th instar show a slight grey tinge. While the *yb* nymphs are characterized



Figs 1–4: The wild-type and *yolk body* mutant specimens of *Pyrrhocoris apterus*. 1 – 5th instar nymph of the wild-type; 2 – imago of the wild-type; 3 – 5th instar nymph of the *yolk body* mutation; 4 – imago of the *yolk body* mutation.

by yolk-like coloration of epidermis (Fig. 3), the *yb* imagoes maintain yolk colour pattern within the first few days of their adult life only. Afterwards, the body coloration, particularly the forewings of the mutant imagoes turn to yolk-orange and then become brick-red (Fig. 4). The mutant phenotype is uniformly expressed in both sexes. No similar developmental change in the body coloration coupled with post-metamorphic aging of imagoes was observed in the wild-type bugs.

Pteridine analyses

Eight different pteridines were identified in the wild-type and the *yb* mutant bugs using TLC and paper chromatography (Table 1): erythropterin I, erythropterin II, D-neopterin, L-neopterin, L-biopterin, violapterin I, isoxanthopterin and 7-methylxanthopterin. Erythropterin II and violapterin I were revealed only by paper chromatography. As no qualitative and quantitative differences in the pteridine patterns between males and females were observed, the pteridine data obtained for both sexes were summed.

TABLE 1. Relative amounts of pteridines in the bodies of *yb* and wild-type bugs, *Pyrrhocoris apterus*: A – whole bodies (TLC analyses); B – eyeless bodies (paper chromatography); C – eyes (paper chromatography).

Sample	Erp I			Erp II			D-Nep			L-Nep			L-Bip			Ixp			7-Mxp			Vip I		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
WT 4d-N	3	3	4	2	3	2	2–3	2	3	2	2	3	3	2–3	3	1	1	1	1	1	1	0	2	0
yb 4d-N	3	3	4	0	1	2	1	2	3	2–3	2	3	3	3	3	1	1	2	2	2	1	0	2	0
WT 2d-I	3	3	4	2	2	2	2	2	2	2	2	4	3	3	3	2	2	2	3	3	2	0	1	0
yb 2d-I	3	3	4	0	0	2	1–2	3	2	2	2	3	3	3	3	2	2	2	2	2	2–3	0	1	0
WT 20d-I	3	3	3	2	2	1	2	3	2	2	2	4	3	3	3	4	4	3	4	4	3	0	1	0
yb 20d-I	2	3	3	0	0	1	2	3	1	1	3	4	3	3	3	2	2	3	3	3	2–3	0	1	0

ABBREVIATIONS: Erp I – Erythopterin I; Erp II – Erythopterin II; D-Nep – D-Neopterin; L-Nep – L-Neopterin; L-Bip – L-Biopterin; Ixp – Isoxanthopterin; 7-Mxp – 7-methylxanthopterin; Vip I – Violapterin I; WT – the wild-type; N – 5th instar nymph; I – imago.

SPOTS SCORING: 4 – very strong fluorescence (very high content of pteridine)
3 – strong fluorescence (high content of pteridine)
2 – medium fluorescence (medium content of pteridine)
1 – weak fluorescence (traces of pteridine)
0 – no fluorescent spot (absence of pteridine)

By TLC, 7 pteridines were identified in the wild-type bugs, but only 6 of them in the *yb* mutants (Table 1). Erythropterin II, occurring in the wild-type bugs was not detected in the bodies of the *yb* mutants, both in the nymphs and imagoes. In contrast with TLC, the paper chromatography permitted the detection of traces of erythropterin II also in the eyeless bodies of the *yb* nymphs, but not in those of the *yb* imagoes (Table 1). However, eyes of both the *yb* and wild-type nymphs and young imagoes revealed medium content of erythropterin II; traces of this pteridine were observed even in the eyes of older (20-day old) imagoes.

Violapterin I was identified only with the use of paper chromatography. It was found to occur in higher quantity only in the nymphs. In imagoes of both the wild-type and the *yb* mutation, this pigment was present only in traces (Table 1). In contrast with age-related

decrease in violapterin I, there was age-related accumulation in isoxanthopterin and 7-methylxanthopterin content in the eyeless bodies and eyes of both the *yb* and wild-type bugs (Table 1). The highest content of these pteridines was found in 20-day old imagoes of the wild type. These results indicate that isoxanthopterin and 7-methylxanthopterin should be degradation products of pteridine metabolism. A slight age-related increase in the content also occurred in the case of D-neopterin, but it was observable in the eyeless bodies of the *yb* and wild-type imagoes only.

No quantitative differences between the *yb* and wild-type bugs were revealed in the case of biopterin. Moreover, the content of this pteridine was maintained at the same level in all ages categories tested (Table 1).

DISCUSSION

A new, *yb* trait described in the present paper is the sixth mutation affecting pteridine biosynthesis in *P. apterus*. It was found to be inherited as an autosomal recessive (Socha, in prep.). The *Pa* and *yb* are the only mutations, which affect coloration of the epidermal cells in the body but do not have any visible effects on eye colour in the mutant nymphs and imagoes. No observable differences between eye colours in *Pa*, *yb* and wild-type bugs have been found.

In contrast with all previously reported body-colour mutations (*wh*, *yw*, *Pa*, *Ap*, *mo* and *m*) of *P. apterus*, the *yb* trait is the only mutation causing the difference in coloration of nymphs and young imagoes, and coloration of older imagoes. A gradual shift from yolk-like coloration to yolk-orange and then to brick-red is accompanied by changes in content of some pteridines and coupled with the ageing of adults. Carotenoids might be also present (e.g., in eyes) but their extraction and analysis require different solvents and developing systems. This is in accordance with the fact that ageing of insects may constitute a factor in pigmentary development (Fuzeau-Braesch, 1985). Age-related change in the *yb* mutation may be connected with sexual maturation and the changes in titre of some hormones occurring during this period of adult life. It is probable that locusts, predominantly *Schistocerca gregaria* and *Locusta migratoria*, provide the best-known case of hormonally controlled colour polymorphism, regulated by the juvenile hormone (JH) (Nijhout & Wheeler, 1982). Regarding JH, it appears not to be involved in age-related colour change in the *yb* mutants of *P. apterus*, as follows from preliminary results with an extirpation of the endocrine gland, corpora allata, in freshly ecdysed imagoes of this mutation (Němec & Socha, unpubl. data). The physiological and endocrinological mechanisms underlying the change in colour pattern observed in the *yb* mutant bugs during the critical developmental period are subjects of the current investigation.

Age-related changes in pteridine compounds and their quantities were reported also in some other insects, e.g., *Stomoxys calcitrans* (L.) (Lehane et al., 1986), *Glossina* spp. (Langley et al., 1988), *Cochliomyia hominivorax* (Coquerel) (Thomas & Chen, 1989) and *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett) (Mochizuki et al., 1993) of Diptera, and *Pectinophora scutigera* (Holdaway) of Lepidoptera (Noble & Walker, 1990). Several of the changes in pteridine levels may be particularly useful for determining the age of field collected insects. However, McIntyre & Gooding (1995) found that accumulation of pteridines depends not only on age but also on diet, the rate of metabolism and sex.

In Heteroptera, investigations concerning pteridines are rather rare. All pteridines identified in Heteroptera belonged to the simple and C-7 substituted pteridines; hydrogenated and C-6 substituted compounds were not mentioned in the most reported species (cf. Melber & Schmidt, 1992). The study of the biosynthetic pathway leading to the synthesis of the main pteridines found in Heteroptera has been hampered in part by the scarcity of mutants affecting pteridine biosynthesis. The body-colour mutants of *P. apterus* have been shown to be a convenient model for such study, particularly for analysis of genetic control of the biosynthesis of pteridine pigments (Socha & Němec, 1992).

Four compounds, i.e., erythropterin, isoxanthopterin, violapterin and 7-methylxanthopterin were revealed in imagoes of the wild-type *P. apterus* (Merlini & Nasini, 1966), and one more pterin in the developing eggs, which was identified as 6-substituted derivate of violapterin (Smith & Forrest, 1969). However, subsequent TLC and paper chromatography analyses of various organs of the wild type of *P. apterus* and of its five body-colour mutants allowed identification of as many as 11 different forms of pteridines (Socha & Němec, 1992). The integument and eyes contained the highest amount of pteridines from all organs studied. The *mo* mutant was the richest in the pigments from all five mutants studied, both in content and variety of pteridines; conversely, the *Pa* bugs were the poorest in this respect. Two groups of the traits have been distinguished regarding the pteridine distribution in the studied organs. The first group involving the wild-type, *mo* and *Ap* mutants, was characterized by the presence of erythropterin, but by absence of xanthopterin and leucopterin in the integument and haemolymph. The second group with *wh* and *yw* mutants was characterized by the presence of xanthopterin (most probably isoxanthopterin) and, in the case of *wh* mutant, also leucopterin, but by a low content or absence of erythropterin. The *Pa* mutation was considered a special case of the first group.

Differences between the qualitative and quantitative data in pteridines described in the present and a previous paper (Socha & Němec, 1992) might resulted from the fact, that we recently used slightly modified methods of extractions, prolonged development of chromatograms in order to distinquish erythropterin I from erythropterin II, and well defined standards. Thus, the xanthopterins I–IV described in our previous paper might represent isoxanthopterin and 7-methylxanthopterin. Violapterin I–IV (Socha & Němec, 1992) could fuse into one spot after prolonged development of chromatograms.

From the present results on pteridine patterns in the wild-type and the *yb* mutation of *P. apterus*, the following conclusions may be made. Erythropterin appears to play an important role in the red coloration of this species. It is probable that the lower content of erythropterin in the eyless bodies of the *yb* bugs, due to lack of erythropterin II, takes part in yolk-coloration of the mutant specimens. This is supported by the occurence of erythropterin II in the *yb* eyes, which remain red-pigmented as in the wild-type. As reported earlier (Socha & Němec, 1992), lower content of erythropterin I was found also in the *wh*, *yw* and *Pa* mutants of this heteropteran. Erythropterin appears to be red pigment occurring in almost all pyrrhocorids, including the *Dysdercus* species, which is in accordance with data obtained from other Heteroptera (Melber & Schmidt, 1992, 1994). According to these authors, even the yellow colouring of the *Dysdercus* species investigated was caused by erythropterin and not by xanthopterin or 7-methylxanthopterin. The yellow, orange, or red colour of these species should be dependent upon the concentration of erythropterin.

In the present study, two more pteridines, biopterin and neopterin were identified in the wild-type and the *yb* mutation of *P. apterus*, until now unknown in this species nor in the Heteroptera. Occurrence of these pigments in *P. apterus* has been revealed also by HPLC (Porcar et al., 1996). According to the pteridine patterns obtained in the present study, the biosynthesis of pteridines in *yb* mutant bugs appears to be inhibited in later steps of the biosynthetic pathways than in the case of the *wh*, *yw* and *Pa* mutations. The present results may be considered as the further step in the identification of the role of individual mutant genes in the biosynthesis of pteridine pigments in heteropterans. However, for more precise identification and quantification of pteridines, better analytical methods (e.g., HPLC, spectral analyses) have to be used.

It is evident that *P. apterus*, with its body-colour mutants, represents a suitable model species for the next in-depth studies about the genetics of the pteridine metabolic pathway.

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