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Comparative analysis of maternal and grand-maternal photoperiodic responses of *Trichogramma* species (Hymenoptera: Trichogrammatidae)

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Abstract. Maternal and grand-maternal photoperiodic responses of *Trichogramma buesi*, *T. embryophagum*, *T. evanescens*, *T. piceum*, *T. principium*, and *T. telengai* were investigated in laboratory conditions. During the experiment, grand-maternal and maternal generations developed at 20°C and one of the 4 photoperiodic regimes: L : D = 12 : 12, 14 : 10, 16 : 8, and 18 : 6 (in total, 16 combinations) while the progeny developed at L : D = 12 : 12 and one of the 3 thermal regimes: 13, 14, and 15°C. The proportion of diapausing individuals in the progeny of all the studied species was significantly dependent on the direct influence of temperature and on the maternal photoperiodic response. The influence of the photoperiodic conditions during development of the grand-maternal generation was statistically significant in 5 of the 6 studied species, being relatively weak in *T. embryophagum* and *T. telengai*, whose geographical ranges extend up to north-western regions of Europe (possibly, these wasps enter diapause so early that the grand-mothers of the diapausing generation develop under long day conditions). Comparative analysis showed that the thresholds of the maternal and grand-maternal photoperiodic responses coincided or almost coincided. The grand-maternal effect was stronger in the progeny of maternal females which developed under short day conditions than in those that developed under long day conditions. This pattern of interaction probably synchronizes the life cycle with seasonal changes because diapause is induced under decreasing day length and thus mothers of diapausing individuals develop at shorter daylength than do grand-mothers. We conclude that the grand-maternal and the maternal effects on *Trichogramma* progeny diapause are based on one and the same photoperiodic response. In nature, the grand-maternal effect increases the proportion of diapausing individuals in the progeny of females which have developed under short day conditions during two generations, thus achieving a "cumulative" photoperiodic effect.

INTRODUCTION

Photoperiod and temperature are well known to be the most common factors inducing winter diapause in temperate zone insect species. These environmental tokens are perceived by the sensitive stage of development which usually precedes the diapausing stage (Tauber et al., 1986; Zaslavski, 1988; Saunders et al., 2002; Danks, 2007; Saunders, 2010). However, the so called "maternal effect" is observed in a number of insect species: the proportion of diapausing individuals depends on the factors that have influenced females of the maternal generation. Moreover, in certain insect species, the tendency to diapause depends on the conditions of development of females of two or even several preceding generations (Lees, 1960; Bogdanova et al., 1978; Zaslavski & Umarova, 1981; Mai Phu Qui & Zaslavski, 1983; Vinogradova, 1991; Reznik & Kats, 2004; Saunders, 2010; Reznik et al., 2012a). While the physiological mechanism and the ecological significance of the maternal influence on the diapause induction have been thoroughly studied in several insect species (Zaslavski, 1978, 1988; Tauber et al., 1986; Mousseau & Dingle, 1991; Mousseau & Fox, 1998; Denlinger, 1998, 2002; Saunders et al., 2002; Danks, 2007), the grand-maternal photoperiodic response has not been sufficiently investigated. In particular, one important question remains: are maternal and grand-maternal effects (if both are present in an insect species) nothing more than different manifestations of the same photoperiodic response or they are based on different and, to some extent, independent reactions? The main aim of the present work was to address this question.

The subjects of our study, minute egg parasitoids in the genus *Trichogramma* are widely used not only for biological control of lepidopteran pests (Smith, 1996) but also as insect models for various eco-physiological studies. In nature, *Trichogramma* species of the temperate zone overwinter as diapausing prepupae. Low temperature is the most important environmental cue that influences *Trichogramma* embryos and larvae inducing the prepupal diapause. In addition, under near-threshold temperatures (12–15°C) the proportion of diapausing individuals usually depends on the photoperiodic conditions during the development of the maternal females (Zaslavski & Umarova, 1981, 1990; Boivin, 1994; Laing & Corrigan, 1995; Reznik et al., 2011a, b). Moreover, in some *Trichogramma* species, a grand-maternal effect has also been observed (Zaslavski & Umarova, 1981; Mai Phu Qui & Zaslavski, 1983; Reznik & Kats, 2004). Both maternal and grand-maternal effects on *Trichogramma* diapause induction are based on long-day photoperiodic response: the proportion of diapausing progeny increases

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Table 1. Natural geographic ranges of the studied *Trichogramma* species and origin of the studied strains.

Trichogramma species	Natural geographic range of the species *	Origin of the studied strain
T. buesi	Southern Europe, Southern Russia, Central Asia	Saratov prov. (Russia)
T. embryophagum	Europe, European Russia, Kazakhstan	Voronezh prov. (Russia)
T. evanescens	Europe, Central and Southern Russia, Southern Siberia, Kazakhstan	Volgograd prov. (Russia)
T. piceum	Southern Europe	Bulgaria
T. principium	Southern Europe, Central Asia	Chimkent prov. (Kazakhstan)
T. telengai	Europe, European Russia, Siberia	Moscow prov. (Russia)

* Data from (Sorokina, 2001) and from the database "Fauna Europea" (http://www.faunaeur.org).

with decreasing daylength. This interaction of maternal and grand-maternal photoperiodic effects and direct thermal responses inhibits diapause induction during spring and early summer, when the daylength is much longer than at the same average temperature during late summer and autumn.

Returning to the main aim of our study, we can advance two working hypotheses.

(1) If the grand-maternal and maternal photoperiodic responses are relatively independent physiological reactions, the threshold day length of the grand-maternal photoperiodic response would be longer than that of the maternal response. This relationship is expected because grand-mothers of diapausing individuals experience longer daylength than do mothers.

(2) For the same reason, we can expect that the grand-maternal photoperiodic response would be stronger (all other conditions being the same) when maternal females develop under the shorter daylengths than grand-maternal females do (or, more generally, the grand-maternal effect would be stronger in the progeny of maternal females developed under the short day conditions).

We have tested these hypotheses using six *Trichogramma* species. In 3 of them (*Trichogramma buesi* Voegele, *T. evanescens* Westw., and *T. principium* Sug. et Sor.) the grand-maternal photoperiodic response was shown to occur (Zaslavski & Umarova, 1981; Mai Phu Qui & Zaslavski, 1983; Reznik & Kats, 2004), while *T. telengai* Sor., *T. embryophagum* Htg., and *T. piceum* Djur. have not been investigated in this respect. Thus, a secondary aim of our investigation was to evaluate the occurrence of the grand-maternal photoperiodic response among *Trichogramma* species.

MATERIAL AND METHODS

The study was conducted with laboratory strains of 6 *Trichogramma* species (Table 1). In the laboratory these strains were cultivated on eggs of the Angoumois grain moth, *Sitotroga cerealella* Oliv. (Lepidoptera: Gelechiidae) during 20–300 generations at temperatures of 20–25°C and day length of 18–20 h, i.e., under the conditions which averted diapause. Immediately before the study, the strains were reared under more strictly controlled diapause averting conditions (20°C, 18L : 6D, and 75% RH) for at least 10 generations. For each experiment, a paper card with 3000–4000 grain moth eggs glued with polyvinyl acetate suspension were subjected for 2 h to parasitization by ca 1000 females of the laboratory line. Then this card with parasitized host eggs (the grand-maternal generation) was separated into 4 parts which were randomly placed in 4 test tubes incubated at 20°C and 75% RH under 4 photoperiodic regimes: L : D = 12 : 12, 14 : 10, 16 : 8, and 18 : 6. At the day of the mass emergence of the grand-maternal generation, a new paper card with 3000–4000 fresh eggs of the grain moth was placed in each tube and subjected to parasitization for 2 h. Then these cards (the maternal generation) were also separated into 4 parts and randomly distributed among the same 4 photoperiodic regimes. Finally, at the day of the mass emergence of the maternal generation, a new paper card with 3000–4000 fresh eggs of the grain moth was placed in each tube, subjected to parasitization for 2 h, and separated into 18 small cards (progeny generation) which were randomly distributed among 3 temperature regimes: 13, 14, and 15°C at 12L : 12D and 75% RH. The above temperature regimens were chosen because they are close to the threshold of diapause induction for all the species under study, as suggested by earlier investigations (Zaslavski & Umarova, 1981, Sorokina & Maslennikova, 1986; Zaslavski & Umarova, 1990; Reznik et al., 2002; Reznik & Kats, 2004; Reznik, 2011; Reznik et al., 2011a, b) and by our (unpubl.) pilot data. Thus, each experiment included 48 treatments, i.e. combinations of 4 "grand-maternal photoperiods" (photoperiodic conditions of development of the grand-maternal generation) with 4 "maternal photoperiods" (photoperiodic conditions of development of the maternal generation) and with 3 thermal regimes of development of the progeny. It is known that in *Trichogramma* the tendency to diapause may vary over generations of a laboratory line even under stable environmental conditions (Zaslavski & Umarova, 1981, 1990; Reznik et al., 2002). Thus, for each *Trichogramma* species, 4 experiments were conducted using different (sequential) generations of laboratory strains. To ensure uniformity, parasitization was always conducted at the same time of the day (between 4 and 6 h after the light-on).

In 10 days after the mass emergence of the non-diapausing fraction of the progeny generation all parasitized host eggs were dissected, diapausing prepupae (each living prepupa was assumed to be diapausing) and non-diapausing individuals (mostly emerged adults, few dead adults inside the host chorion, and sporadic pupae) were recorded. As *Trichogramma* females usually lay only one egg in each egg of the grain moth, the number of emerged adults was estimated by the number of parasitized eggs with emergence holes. The few (less than 1–2%) individuals that died during the larval or prepupal stages were excluded from consideration. Then the percentage of diapausing individuals was separately calculated for each small card of the progeny generation (50–100 host eggs parasitized during 2 h by a random sample from about 500 females) that was considered as a replication for the statistical treatment. Thus, the experiment included 6 replicates for each of the 48 experimental treatments conducted with each of the 4 generations of each of the 6 *Trichogramma* species (a total of 6912 cards with more than 400,000 parasitized host eggs).

Medians and quartiles of untransformed data (percentages) were used as descriptive statistics. For further statistical treatment, proportions of diapausing individuals were arcsine – square root transformed and analysed with ANOVA. It is known

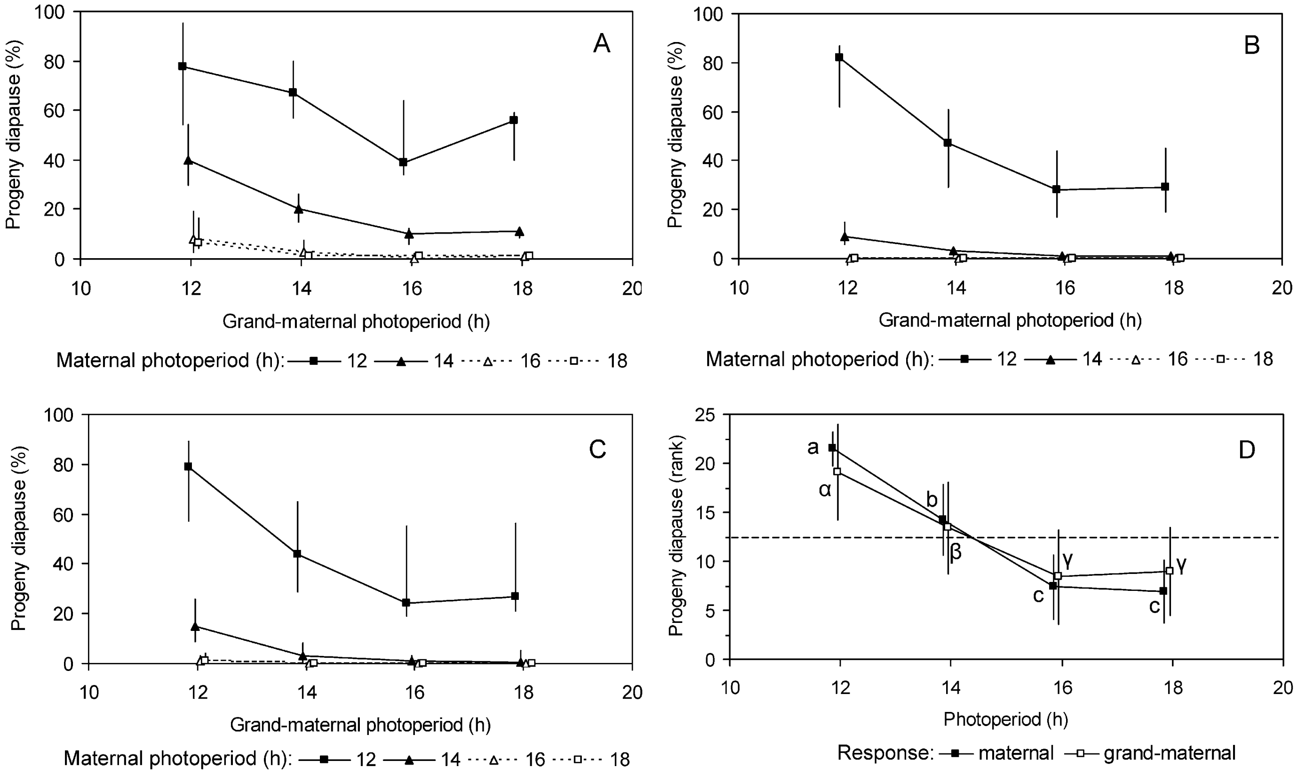

Fig. 1. Maternal and grand-maternal photoperiodic responses of *Trichogramma buesi* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

(see Introduction for the references) that maternal and grand-maternal photoperiodic responses in *Trichogramma* wasps can be revealed only when the progeny develop under temperatures close to the threshold of the diapause induction and the proportion of diapausing progeny strongly depends on temperature. However, it has been recently demonstrated (Reznik et al., 2012b) that although the impact of the maternal photoperiodic response can be revealed only within a very narrow thermal range, the relative strength of the diapause-inducing effect of different day lengths is independent of the temperature regimen of the progeny development. Thus, to reveal the general species-specific patterns of the maternal and the grand-maternal photoperiodic responses, the data were ranked in two ways. First, the proportions of diapausing progeny induced by different grand-maternal photoperiods (i.e. photoperiodic conditions of the developing grand-maternal generation) were separately ranked for each combination "temperature * maternal photoperiod * *Trichogramma* species" and then the ranks were pooled for each species and plotted against grand-maternal photoperiods. Second, the proportions of diapausing progeny induced by different maternal photoperiods (i.e. photoperiodic conditions of the developing maternal generation) were separately ranked for each combination "temperature * grand-maternal photoperiod * *Trichogramma* species" and then the ranks were pooled separately for each species and plotted against maternal photoperiods. These pooled ranked data for each species were analysed with one-way ANOVA and the Tukey HSD test. All the calculations were made with SYSTAT.

RESULTS

As seen in Table 2, the proportion of diapausing individuals was strongly dependent on the sum of the experi-

TABLE 2. Relative strength of the influence of temperature, grand-maternal photoperiod, maternal photoperiod, and difference between generations on the proportion of diapausing progeny of *Trichogramma* species (multiple correlation coefficient R, F – ratios, and their significance calculated by 4-way ANOVA of arcsine-transformed data: *** – $p < 0.001$, ** – $p< 0.01$, * – $p < 0.05$, n.s. – not significant).

Trichogramma species	Multiple R		F-ratios							
			Temperature		Grand-maternal photoperiod		Maternal photoperiod		Generation	
T. buesi	0.985	***	766.4	***	765.0	***	7779.2	***	233.6	***
T. embryophagum	0.883	***	624.2	***	1.8	n.s.	209.2	***	318.1	***
T. evanescens	0.977	***	6352.9	***	57.7	***	1360.1	***	145.1	***
T. piceum	0.960	***	3019.7	***	23.1	***	827.4	***	413.1	***
T. principium	0.985	***	1633.9	***	377.7	***	7335.0	***	164.8	***
T. telengai	0.957	***	1596.5	***	3.8	**	1943.4	***	75.0	***

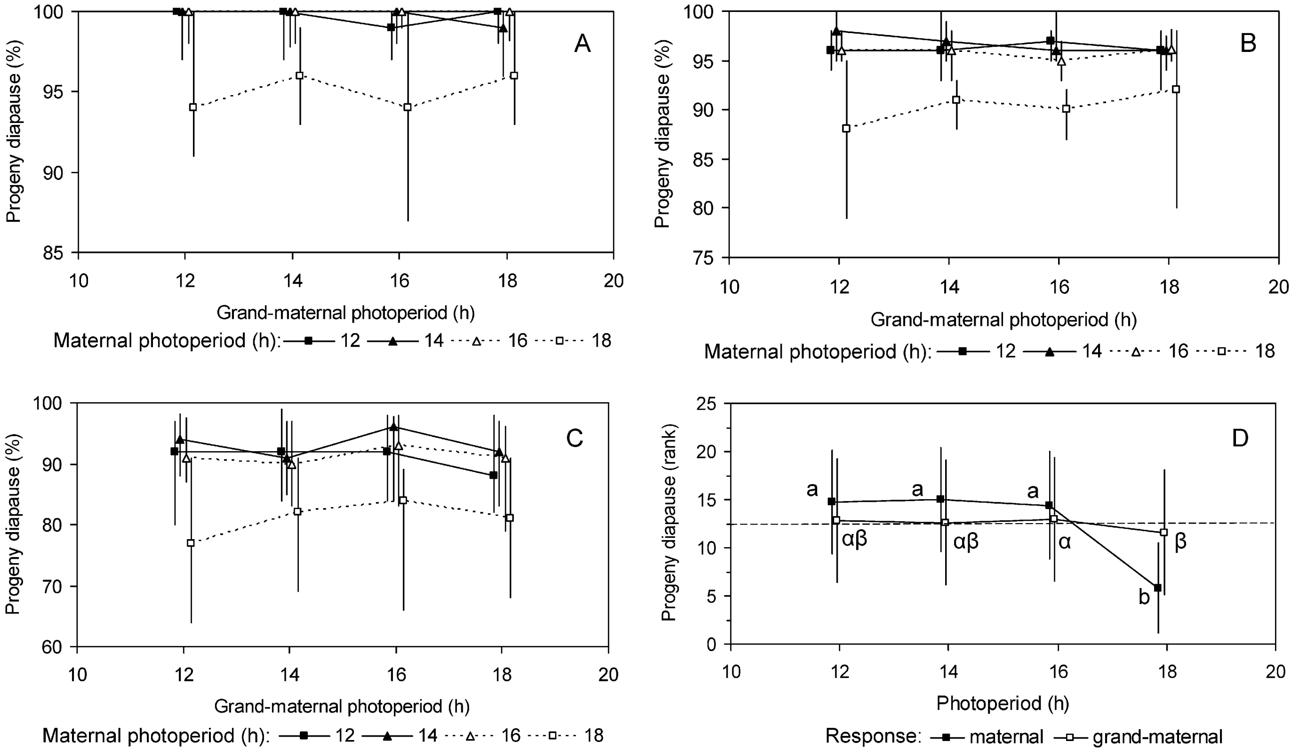

Fig. 2. Maternal and grand-maternal photoperiodic responses of *Trichogramma embryophagum* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

mental factors: the multiple correlation coefficients R varied from 0.883 (*T. embryophagum*) to 0.985 (*T. buesi* and *T. principium)*. In particular, the direct influence of temperature and the maternal photoperiodic response were the strongest factors, the difference between generations was not as strong, although always highly significant, while the grand-maternal photoperiodic response was significant in 5 of the 6 studied *Trichogramma* species. In combination, thermal and photoperiodic responses produced a very strong impact: as seen in Figs 1–6 (A–C parts), the difference between treatments (combinations of photoperiodic regimes of development of the grand-maternal and maternal generations and thermal regime of development of the progeny) in most of the studied species reached 80–90%. In *T. telengai* (Fig. 6) and in *T. embryophagum* (Fig. 2) the maximal difference between treatments was smaller because of a strong tendency to diapause which was recorded even under most "diapause-averting" treatments (grand-maternal and maternal females have developed at long day and progeny developed at high temperature).

Interactions of the above factors were also significant with only a few exceptions (Table 3). Note that all non-significant interactions included grand-maternal photoperiod and in 4 of the 5 cases they were recorded for *T. embryophagum* and *T. telengai.* From Figs 1–6 (A–C parts) it is seen that in most *Trichogramma* species developed at most of the temperature regimes used in our study the relative strength of the grand-maternal photoperiodic response markedly depended on the maternal photo-

Table 3. Relative strength of the interactions between temperature, grand-maternal photoperiod, maternal photoperiod, and difference between generations in their influence on the proportion of diapausing progeny of *Trichogramma* species (F-ratios and their significance calculated by 4-way ANOVA of arcsine-transformed data: *** – $p < 0.001$, ** – $p < 0.01$, * – $p < 0.05$, n.s. – not significant).

Trichogramma species	Temperature and grand-maternal photoperiod		Temperature and maternal photoperiod		Temperature and generation		Maternal photoperiod and grand-maternal photoperiod		Generation and grand-maternal photoperiod		Generation and maternal photoperiod	
T. buesi	13.8	***	36.2	***	186.9	***	67.8	***	26.5	***	93.9	***
T. embryophagum	1.0	n.s.	3.9	***	47.4	***	2.3	*	1.4	n.s.	4.0	***
T. evanescens	8.2	***	99.0	***	135.3	***	1.3	n.s.	2.8	**	57.4	***
T. piceum	3.5	**	41.2	***	120.8	***	3.0	**	2.0	*	23.3	***
T. principium	29.5	***	207.0	***	92.3	***	41.0	***	14.8	***	33.8	***
T. telengai	1.1	n.s.	68.8	***	64.4	***	3.5	***	1.3	n.s.	11.0	***

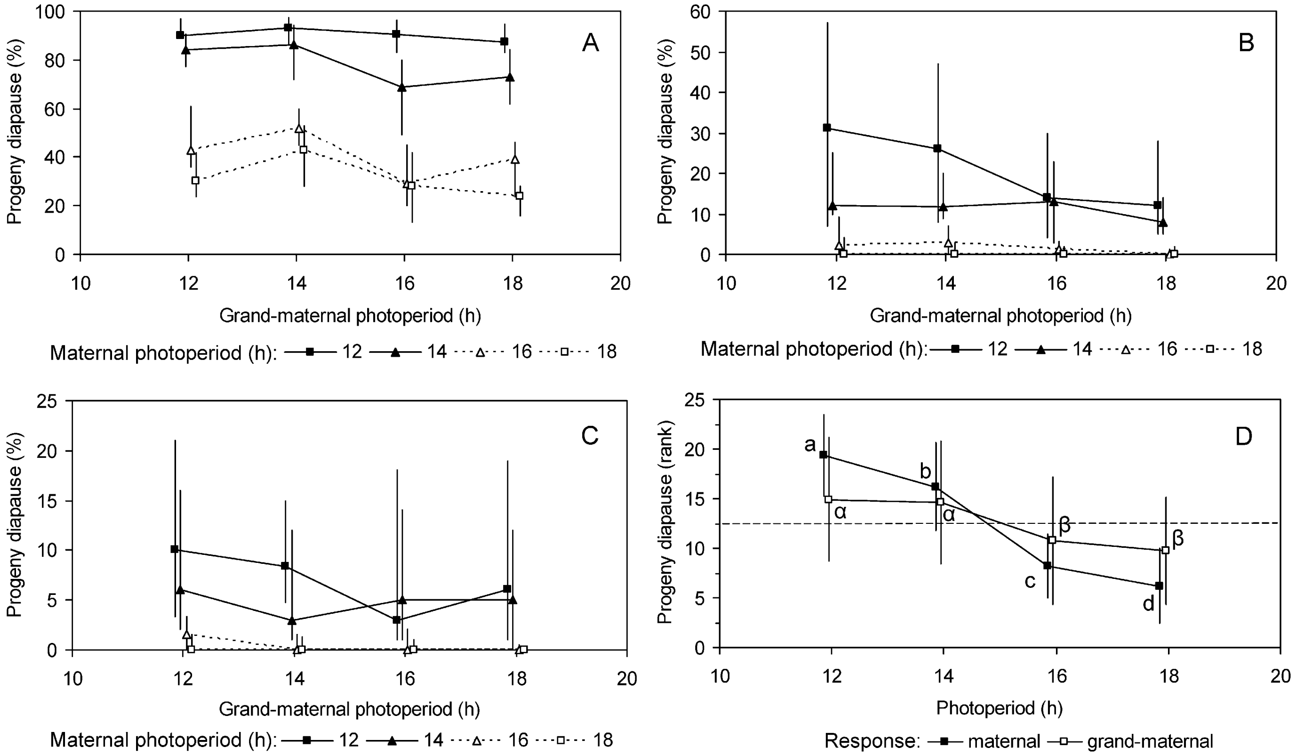

Fig. 3. Maternal and grand-maternal photoperiodic responses of *Trichogramma evanescens* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

period. To measure this effect, the data for different maternal photoperiods were separately analysed by 3-way ANOVA (grand-maternal photoperiod, temperature, and generation were the factors). As seen in Table 4, with an increase in the maternal photoperiod, the relative strength of the influence of the grand-maternal photoperiod on the proportion of diapausing progeny markedly decreased, while the direct influence of temperature did not show any clear trend. To reveal this general tendency, the *F*-ratios from Table 4 were separately ranked for each *Trichogramma* species and then the ranks for different species were pooled. The results of this analysis (Fig. 7) suggested that the mean relative strength of the grand-maternal photoperiodic response significantly decreased with the maternal photoperiod, while the strength of the direct thermal response did not show any significant changes.

As was noted above (Table 2, Figs 1–6) the proportion of diapausing progeny was markedly dependent on all of the experimental factors which we have used. Thus, to reveal the species-specific patterns of the maternal and the grand-maternal photoperiodic responses, the data were ranked and plotted against maternal and grand-maternal photoperiods (see Material and Methods for the detailed description of this transformation). Both types of graphs are shown in parts (D) of Figs 1–6. It is clearly seen that the visually estimated thresholds varied from 14.5 h in *T. buesi* (Fig. 1) to more than 16 h in *T. embryophagum* (Fig. 2), but the thresholds of the maternal and the grand-maternal photoperiodic responses of *T. buesi, T. embryophagum*, and *T. piceum* practically coincided (Figs 1, 2, 4). In *T. evanescens* (Fig. 3) and *T. principium* (Fig. 5), the threshold day of the grand-maternal response was slightly longer than that of the maternal response, but

Table 4. Relative strength of the influence of grand-maternal photoperiod and temperature on the proportion of diapausing progeny in relation to maternal photoperiod and *Trichogramma* species (F-ratio and its significance calculated by ANOVA of arcsine-transformed data: *** – $p < 0.001$, ** – $p< 0.01$, * – $p < 0.05$, n.s. – not significant).

Maternal photoperiod	12 : 12		14 : 10		16 : 8		18 : 6		12 : 12	14 : 10	16 : 8	18 : 6
Trichogramma species	Influence of grand-maternal photoperiod								Influence of temperature (p < 0.001 in all cases)			
T. buesi	517.3	***	212.9	***	63.7	***	75.6	***	126.1	356.1	159.5	142.4
T. embryophagum	3.3	*	3.5	*	0.9	n.s.	1.7	n.s.	192.5	136.9	152.0	159.7
T. evanescens	11.1	***	13.7	***	41.6	***	10.0	***	1711.9	1965.9	2317.4	899.2
T. piceum	8.0	***	14.9	***	3.5	*	1.4	n.s.	725.3	939.4	721.8	723.9
T. principium	190.9	***	129.7	***	38.0	***	37.0	***	868.9	632.2	116.2	130.9
T. telengai	7.2	***	4.1	**	1.4	n.s.	1.2	n.s.	142.2	326.1	441.1	858.3

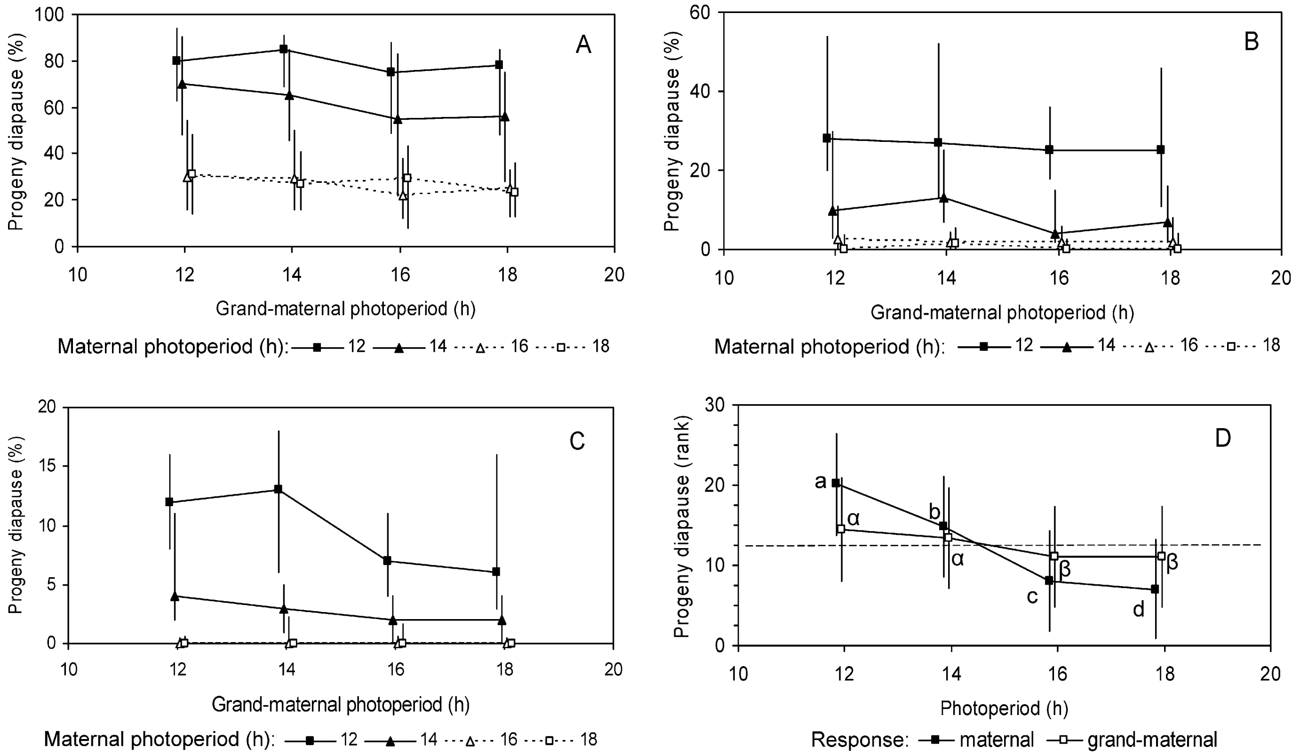

Fig. 4. Maternal and grand-maternal photoperiodic responses of *Trichogramma piceum* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

considering the scale of the graph (2 h) and the high variability of the results, this small (less than 30 min) difference was not significant.

DISCUSSION

Direct thermal and maternal photoperiodic effects revealed in our experiments have been demonstrated earlier for the studied species as well as for several other representatives of the genus *Trichogramma* (Bonnemaison, 1972; Zaslavski & Umarova, 1981, 1990; Mai Phu Qui & Zaslavski, 1983; Sorokina & Maslennikova, 1986; Boivin, 1994; Laing & Corrigan, 1995; Garcia et al., 2002; Reznik et al., 2002; Reznik & Kats, 2004; Ma & Chen, 2006; Pizzol & Pintureau, 2008; Sorokina, 2010; Reznik, 2011), although the diapause-inducing effect of a wide range of photoperiods in combination with a range of thermal regimes has been studied only in *T. piceum* (Reznik et al., 2012b). Similar interaction of direct thermal and maternal photoperiodic responses has been found also in certain other hymenopteran parasitoids from families Aphidiidae (Brodeur & McNeil, 1989; Polgár & Hardie, 2000), Braconidae (Ryan, 1965; Kenis, 1997; Li et al., 2008), and Eulophidae (Milonas & Savopoulou-Soultani, 2000).

A significant generational effect that was revealed in our study, also has been demonstrated earlier (Zaslavski & Umarova, 1981, 1990; Reznik et al., 2002). Similar variations in the tendency to diapause have been observed in the blowfly *Calliphora vicina* R.-D., which is also a multivoltine insect with a strong maternal effect (Bogdanova et al., 1978; Zaslavski, 1978, 1988; Vinogradova, 1991; Denlinger, 1998). Zaslavski (1988) conceived that this variability is not connected with selection (as suggested, in particular, by the cyclicity of changes) but it is correlated with the maternal effect and may be based on the same mechanism. As for the maternal effect on diapause, its mechanisms may differ in different insects (Mousseau & Dingle, 1991; Mousseau & Fox, 1998; Denlinger, 1998, 2002; Saunders et al., 2002; Saunders, 2010). The results of our recent study (Reznik et al., 2012a) suggest that maternal and grandmaternal photoperiodic effects in *Trichogramma* are probably based on the epigenetic inheritance in the strict sense of the term, i.e. on the transgenerational transmission of variations in DNA expression. On the other hand, the generational effect can be connected with circannual rhythms (Saunders et al., 2002). Note that high intergenerational variability in fecundity, parasitization activity, and other biological parameters has been also reported for *Trichogramma* females (Reznik et al., 1996; Schmuck et al., 1996; Hoffmann et al., 2001).

As clearly seen from Figs 1–6, the maternal effect on the progeny diapause in all studied species was manifested as a long-day photoperiodic response but the threshold day length varied from ca 14 h in *T. principium* and *T. buesi* to ca 16 h in *T. embryophagum* and *T. telengai*. This difference can be possibly explained by that *T. embryophagum* and *T. telengai* are widely distributed over Europe and European Russia including Central and North-Western regions, while *T. principium* and *T. buesi*

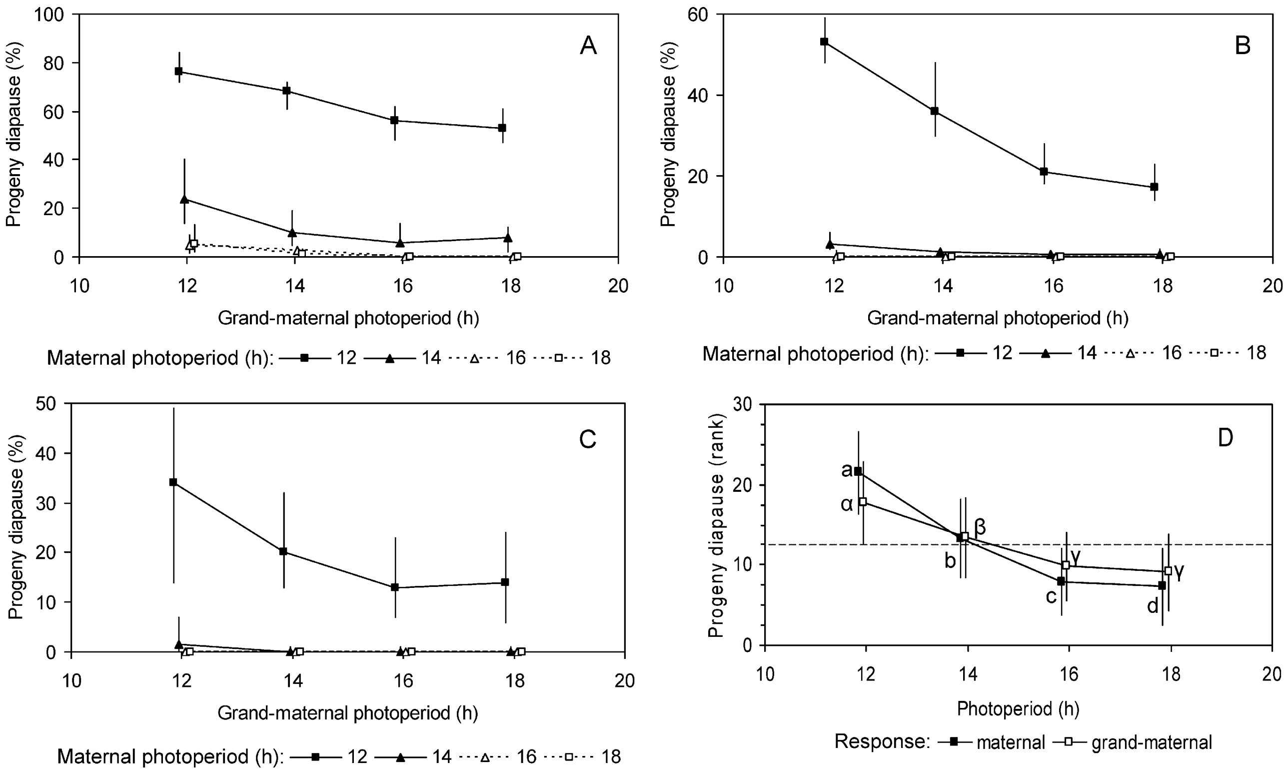

Fig. 5. Maternal and grand-maternal photoperiodic responses of *Trichogramma principium* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

occur only in Southern Europe, Southern Russia, Southern Kazakhstan, and Central Asia (Table 1), where the optimal time to enter diapause coincides with much shorter days. This latitudinal variation in threshold of photoperiodic response has been found in many insect taxa (Tauber et al., 1986; Zaslavski, 1988; Saunders et al., 2002, Danks, 2007; Saunders, 2010).

Analyzing the results of this study it should be remembered that not photoperiod but temperature is the main diapause-inducing factor in *Trichogramma* species. Moreover, in some *Trichogramma* species inhabiting northern Europe (St. Petersburg province) the maternal photoperiodic response was not detected and diapause seemed to be regulated only by temperature signals (Sorokina, 2010). The threshold of the direct thermal response exhibits wide intraspecific variation, but usually temperatures of 10–12°C are optimal for diapause induction, temperatures of 17–20°C and higher stimulate active development independently of day length, while temperatures of 8°C and lower lead to cold quiescence rather than to diapause. The maternal photoperiodic response of *Trichogramma* species can be usually revealed at temperatures of 13–15°C (Zaslavski & Umarova, 1981, Mai Phu Qui & Zaslavski, 1983; Sorokina & Maslennikova, 1986; Zaslavski & Umarova, 1990; Boivin, 1994; Laing & Corrigan, 1995; Garcia et al., 2002; Reznik et al., 2002; Reznik & Kats, 2004; Ma & Chen, 2006; Pizzol & Pintureau, 2008; Sorokina, 2010; Reznik, 2011; Reznik et al., 2011a, b, 2012a, b). This photoperiodically based maternal modification of the thermal response avoids the untimely induction of diapause in response to occasional cold waves in spring – early summer, when the temperatures of 13–15°C are combined with much longer days than in autumn. Thus, under natural conditions, the induction of diapause is synchronized both with the astronomical season (the photoperiodic response) and with the peculiarity of a given year (the thermal response).

The grand-maternal effect was statistically significant in 2 of the 3 newly investigated species. In combination with the earlier published data (Zaslavski & Umarova, 1981; Mai Phu Qui & Zaslavski, 1983; Reznik & Kats, 2004) this result suggests that the influence of the grand-maternal photoperiod on the diapause incidence in their grand-progeny is rather a rule than an exception in *Trichogramma* wasps, although the relative importance of this response may vary greatly from species to species. It is noteworthy that the grand-maternal effect was the strongest in *T. buesi* and *T. principium* (which, as was noted above, inhabit only the south of Palearctic) and the weakest in *T. embryophagum* and *T. telengae* whose geographical ranges extend relatively far to the north. Possibly this tendency may be explained by that in the northern temperate zone *Trichogramma* wasps develop slowly and enter diapause early, and thus the grand-mothers of the diapausing generation develop under long day conditions, but the number of the studied species is not yet sufficient for a certain conclusion.

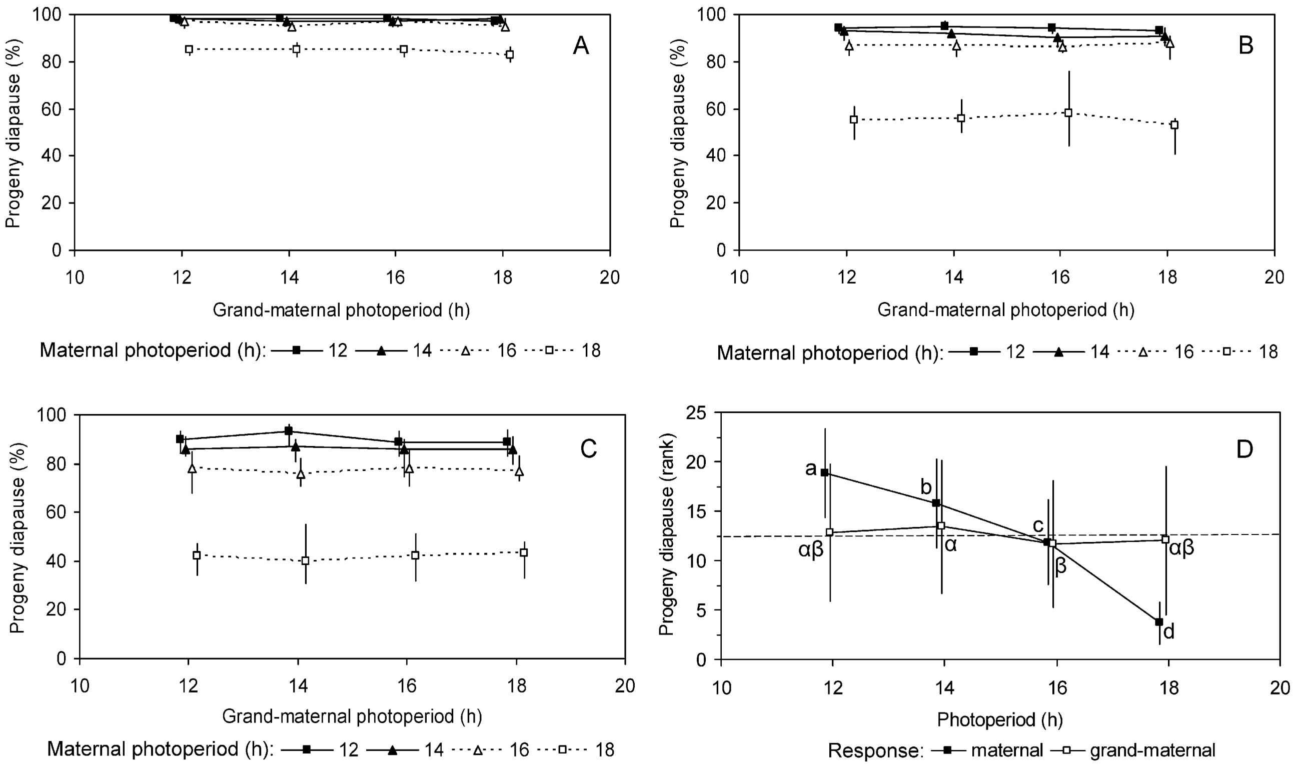

Fig. 6. Maternal and grand-maternal photoperiodic responses of *Trichogramma telengai* at different thermal regimes of the progeny development. A, B, and C – the progeny developed at 13, 14, and 15°C, respectively, medians and quartiles are shown. D – the pooled ranked data for all temperatures, means and SD are shown, means indicated by different Latin (for the maternal response) or Greek (for the grand-maternal response) letters are significantly different ($p < 0.05$ with the Tukey HSD test).

The so called "stepwise photoperiodic reaction" or the response to the direction of change in the length of the day has been observed in several insect species (Tauber et al., 1986; Zaslavski, 1988; Saunders et al., 2002). Indeed, a slight but statistically significant effect of the change in photoperiod was demonstrated for *T. principium* (Reznik & Kats, 2004). However, in the present study, this effect was not recorded: when grand-mothers developed at longer day than mothers did, the proportion of diapausing progeny was not higher than when both mothers and grand-mothers developed at the shorter of the two photoperiods. This contradiction, however, can be explained by that in the present study we used "natural" day lengths from 12 to 18 h, while the stepwise photoperiodic reaction in *T. principium* was detected only under ultra-short photoperiods (Reznik & Kats, 2004). Note that in these experiments photoperiod differed between generations but was always constant during development of a given generation. In the other studies on the insect response to the changing day length, the changes occurred within one generation (Tauber et al., 1986; Zaslavski, 1988; Saunders et al., 2002). Experiments of this kind have been also conducted with *Trichogramma* wasps, the stepwise photoperiodic response was very weak (Ivanov & Reznik, 2008) or was not significant (Reznik et al., 2011b).

Returning to the main aim of our study, we conclude that the first hypothesis was not supported by the experimental data: the thresholds of the maternal and the grand-maternal photoperiodic responses of the studied species coincided or almost coincided. It may be speculated that experiments with finer scale of day length could demonstrate small differences between maternal and grandmaternal thresholds. However, considering that the prepupal diapause in *Trichogramma* wasps can be induced only at temperatures of 14–15°C or lower, the development of the preceding (maternal) generation in the natural conditions lasts about a month. At the latitudes of 40–50° (where the species with the strong grand-maternal effect occur) in the end of summer–autumn the decrease in the daylength during one month is 1–2 h. Thus, the corresponding ecologically significant difference between thresholds of photoperiodic responses, if it exists, would have been detected in our experiments. Hence, we conclude that a difference between the thresholds of the maternal and the grand-maternal photoperiodic responses of the studied *Trichogramma* species is absent or at least it has little or no ecological value.

Our second hypothesis, on the contrary, was confirmed by the experiments. The relative effect of the grand-maternal photoperiod on the proportion of diapausing progeny markedly decreased with increasing maternal photoperiod (Table 4, Fig. 7). Note that this trend cannot be explained (or can be only partly explained) by the strong diapause-averting effect of the long maternal photoperiod which inhibits the induction of diapause and thus suppresses the manifestation of any other photo-thermal response. First, the relative strength of the direct thermal response was not significantly dependent on the photope-

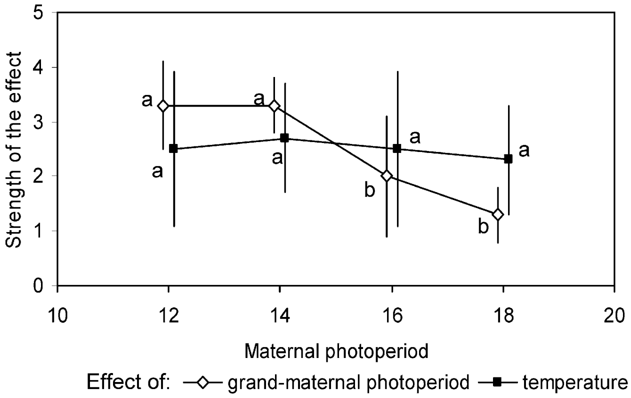

Fig. 7. Influence of photoperiodic conditions of development of the maternal generation on the relative strength of the grand-maternal photoperiodic response and of the direct thermal response. Means and SD of ranked F-ratios (see Table 4) are shown. Means indicated by different letters along the same line are significantly different ($p < 0.05$ with the Tukey HSD test).

riodic conditions of development of the maternal females. Second, the decrease in the strength of the grand-maternal effect was observed not only when the long maternal photoperiod reduced the proportion of diapausing progeny to less than 10–20% (Figs 1, 4, and 5) but also when the percentage of diapausing individuals moved towards 50% (Figs 2 and 6). Theoretically it could be expected that the "threshold zone" of the maternal photoperiodic response is most sensitive to the "grand-maternal correction". This assumption is suggested, for example, by the analogy with the maternal photoperiodic correction of the direct thermal response which in *Trichogramma* species can be detected only at near-threshold temperatures (Zaslavski & Umarova, 1981, 1990; Boivin, 1994; Reznik, 2011). Similarly, the thermal correction of the photoperiodic response is often strongly manifested near the photoperiodic threshold (Tauber et al., 1986; Zaslavski, 1988; Saunders et al., 2002). Generally, the maximum sensitivity zone (the maximum derivative) of a sigmoidal "dose-response" curve also occurs at the 50% level.

As was noted in the Introduction, only a few studies have been devoted to ecological or physiological aspects of the grand-maternal photoperiodic effect in insects. In particular, endogenous circannual rhythms which have been found in several insect species from different taxa are undoubtedly based on the transgenerational transfer of information. These rhythms can be entrained by seasonal changes in day length but this phenomenon differs essentially from the grand-maternal photoperiodic response (Zaslavski, 1988; Saunders et al., 2002; Danks, 2007; Saunders, 2010). The "interval timer" that extends over several generations and inhibits the diapause-inducing effect of short day conditions in the aphid *Megoura viciae* Buckton (Lees, 1960; Saunders et al., 2002, Saunders, 2010) is somewhat similar to the transgenerational photoperiodic response. However, this reaction is triggered by photoperiod not directly but via the diapause induction (response to short days is blocked in the progeny of aphids which emerged from overwintered eggs). Larval diapause in the blowfly *Calliphora vicina* R.-D., a very popular insect model for various experimental studies, was shown to be regulated by interaction of direct and maternal responses to the thermal and photoperiodic conditions of the environment (Vinogradova, 1991; Denlinger, 1998, 2002; Saunders et al., 2002; Saunders, 2010) and these reactions seem to be very similar to those described for *Trichogramma* species (Reznik, 2011). Particularly, multigenerational laboratory experiments suggested that direct and maternal photoperiodic responses of *C. vicina* are based on one and the same physiological mechanism (Bogdanova et al., 1978). Based on the results of that study, Zaslavski (1978) also reasoned that in the natural conditions the adaptation to the seasonal changes of the climate results from the interaction of photothermal responses of the sequence of generations.

Summarizing the results of our study we conclude that the grand-maternal and the maternal effects on *Trichogramma* progeny diapause are most probably based on one and the same photoperiodic response and from this aspect the grand-maternal effect can be considered as a simple continuation of the maternal effect to the next – and the following (Reznik et al., 2012a) – generations. However, the relative strength of this transgenerational impact varies markedly among *Trichogramma* species and depends significantly on the photoperiodic conditions of development of the maternal generation. The grand-maternal effect might conceivably increase the proportion of diapausing individuals in the late autumn, in the progeny of females which have developed under short day conditions during two and more generations, thus achieving a "cumulative" photoperiodic response.

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