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Effect of Different Temperatures on Colony Characteristics of *Bombus terrestris* (Hymenoptera: Apidae)

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Key words
***Bombus terrestris*, Colony initiation, Colony development, Rearing, Temperature.**

ABSTRACT

In the present study, effect of five different temperatures (24 ºC, 26 ºC, 28 ºC, 30 ºC and 32 ºC) was studies on different colony characteristic of post-hibernated queens of *Bombus terrestris* during flight activity period and solitary phase of development. Results showed that survival rate and colony initiation rate of the queen was highest at 28 ºC and 30 ºC. Different parameters in the initial stage of colony growth like pre-oviposition period and emergence timing of the first worker were observed best at 28 and 30 ºC. Number of workers produced in the first brood and in the total lifespan of the colony was also observed higher at 28 ºC. Higher number of progeny queens was produced at temperature treatment of 30 ºC. Therefore, 28-30 ºC temperature exposure to the post-hibernated queens was found the best for artificial rearing of *B. terrestris* and developing strong colony characteristics.

INTRODUCTION

Pollinators provide key ecosystem services that empower plant to produce fruits and seeds. Bumblebees are most important pollinators of agriculture both in open field conditions and protected farming systems (Corbet *et al*., 1991; Ahmad *et al*., 2015). These pollinators bees are often described as primitively eusocial insects which possess 300 species. Latest classifications place all of the identified species in a single genus *Bombus* meaning 'booming' (Prys-Jones and Corbet, 1991). These are mainly confined to temperate regions of the world due to their certain temperature requirement (Williams, 2007).

Greenhouse tomato (*Solanum lycopersicum* L.) is the key crop pollinated by bumblebees globally (Dogterom *et al*., 1998; Morandin *et al*., 2001; Winter *et al*., 2006). Until present, pollination of glasshouse tomatoes has been carried out by hand with a vibrating wand, no doubt a very tedious job and expensive in terms of labour (Cribb, 1990). Worldwide, about 95% of bumblebee sales are for greenhouse tomato which comprises a more than 40, 000 hectares of greenhouse crops. Tomato crop in greenhouse culture is usually grown all the year which require more than 50 bumblebee hives per hectare. The value of these bumblebee pollinated tomato crops is estimated to be € 12000 million per year (Velthuis and van Doorn, 2006).

On a commercial scale, bumblebee rearing started in 1987 and has been available in portable boxes for crop pollination (Mitsuhata, 2000). Worldwide there are about more than 30 Bombi-culture industries but most of the market share is captured by three companies. Koppert Biological Systems (Netherland), Biobest (Belgium) and BBB (Bunting Brinkman Bees, Netherlands) are the biggest producers of bumblebee in the world (Velthuis and van Doorn, 2006).

European bumblebee, *Bombus terrestris* is the most preferred species for bumblebee breeder. It is easily available in Europe where the technique for year round rearing and commercial production has evolved (Velthuis and van Doorn, 2006). Large colony size, wide distribution, adaptability to diverse climatic condition, habitats and flower types makes it a hardy and efficient pollinator favoured in commercial rearing (Velthuis and van Doorn, 2006). It can visit 20 to 50 simple, small flowers per minute and in the case of red clover between 20 to 35 flowers per minute (Free, 1993).

Bombus terrestris is univoltine in nature and its life cycle has two phase (i) solitary phase and (ii) social phase. *B. terrestris* colonies are comprised of different caste for their reproductive, foraging, defence and other tasks necessary for their survival. In the solitary phase of their life cycle, queens enter the diapauses after successful

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mating in fall and after emergence from diapauses in spring, queens start foraging and looking for appropriate nest sites for oviposition. In the social phase of development, the majority of tasks of colony performed by the emerging workers and queen perform mostly reproductive work. Towards the end of colony cycle, young queens and males are produced. After successful mating young queen enters into the diapause and rest of the colony collapses (Lopez *et al*., 2009; Prŷs-Jones and Corbet, 2011).

Different factors like rearing conditions, nest material and diet play important role in artificial rearing of this important pollinator (Yoon *et al*., 2002, 2005; Imran *et al*., 2017). Commercial scale rearing of bumblebees requires efficiency in rearing processes to enhance their utilization for the benefit of farmers. Temperature plays an important role in the regulation of growth and development and affecting almost every aspect of life (Obrycki and Tauber, 1981; Srivastava and Omkar, 2003; Omkar and Pervez, 2004; Pervez and Omkar, 2004). Life duration, oviposition and diapause are some most important aspects which are under the influence of temperature changes. Change in temperature from ambient level showed marked variation in egg development time of *Sitotroga cerealella* (Oliver) (Perez-Mendoza *et al*., 2004). Similarly, oviposition rate of *Periplaneta japonica* and diapause termination in bumblebees showed temperature influence with desired development under ambient level for different life stages need (Alford, 1969; Tanaka and Uemura, 1996).

Oviposition and production of sexuals of bumblebees have shown the influence of temperature (Tasei, 1994; Yoon *et al*., 2002). Previous studies have recommended the different range of temperature for artificial rearing of the bumblebee. The present study was planned with the objective to find favourable exposure temperature for the survival of the post- hibernated queens after flight activity period and their impact on colony initiation and further colony development.

MATERIALS AND METHODS

Experimental insect

Post-hibernated queens of *B. terrestris* were used in this experiment. Queens were collected from the mass rearing system of Non-Apis Bee Laboratory, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi. During activation period of one week, queens were subjected to the flight cages (40×40×60 cm) under white light conditions. The ground of flight cage was covered with filter paper and provided with sugar syrup and pollen as a diet source of queens.

Temperature treatments

There were five different temperature treatments that were used in this experiment that include 24 ºC, 26 ºC, 28 ºC, 30 ºC and 32 ºC. Queens in these cages were randomly subjected to one of the temperature treatment and relative humidity for all the five temperatures was maintained at 60±10%. Temperature and relative humidity for all the applied treatments were checked on a daily basis by using a thermo-hygrometer.

Colony initiation and development

After one week of flight activity in cages, those queens who rubbed their abdomen on the base of flight cages were transferred to the small transparent plastic box. The ground of the starter boxes was covered with cardboard material. To stimulate oviposition, queens in the starter boxes were provided with previously frozen dead pupa that had been horizontally fixed with glue on the ground of cardboard material (Kwon *et al*., 2003; Gurel and Gosterit, 2008), and two small sized helper workers of the *Bombus terrestris* were also provided to the queen in starter box.

Starter boxes were again subjected to the respective temperature treatment chamber. Pollen and sugar solution were provided to the queens in the starter boxes on daily basis. Queens in the starter boxes were monitored on daily basis for observation of colony initiation time.

Queens started egg lying on pupa or on the ground of cardboard material and workers helped the queen in the initiation of egg lying. Helper workers were removed/replaced every week until the queen started egg laying. When queens started oviposition, helper workers were removed. Starter boxes remained in the relevant temperature condition until the emergence of first brood workers.

Colonies were shifted to the larger boxes after the emergence of worker of the first batch. The larger colony boxes were connected with sugar solution container via wick present at the bottom. Rest of colony development was completed in larger boxes in a controlled room (Temperature = 27 ºC; Relative humidity = 60±10%) in complete darkness except when colonies were being observed. Illuminated red light with minimum light intensity at which observation was possible to observe different life history parameters (bumblebees are colour blind to red light).

Observation of parameters

Following parameters were observed in this study: (i) Survival rate and colony initiation rate of the queens. (ii) Pre-oviposition period. (iii) Number of egg cells in first brood. (iv) First brood size. (v) Emergence timing of first worker, males and progeny queen in a colony. (vi)

Switch point and Competition point. (vii) Total number of workers, males and progeny queens produced in a colony. (viii) Lifespan of founding queen, and (ix) Energy spent on sexuals was calculated by using the following formula:

Energy spent on sexuals =

(No. of queens × 7.83 kJ) + (No. of males × 2.35kJ)

As 7.83 kJ and 2.35 kJ of energy are required to produce one queen and male, respectively (Beekman and van Stratum, 1998).

Data analysis and statistic

Data of different life parameters were analyzed using analysis of variation and means were compared with t-test at 5% probability. Chi-squares analysis was performed using SPSS programs (Norus, 2006).

RESULTS

Survival rate in flight cage

Figure 1 shows the survival and colony initiation rate of queens, pre-oviposition periods, production of egg cells and brood size in first batch, emergence timing of first worker, male and daughter queen, and total number of workers, males and progeny queens produced for *Bombus terrestris*.

Highest survival rate of queens was observed at 28 ºC with 90% queens and the lowest at 24 ºC with 74% queens (Fig. 1A). Temperature above and below 28 ºC resulted in decreased progeny queens inside flight cage kept for flight activity period. The highest colony initiation rate was observed at 30 ºC (79%) while it was lowest at 24 ºC (67%).

Significant difference existed for pre-oviposition period of *B. terrestris* queens at five different temperatures ranging 24-32 ºC (Fig. 1B). Shortest pre-oviposition period (6.1±1.15 days) was observed at 30 ºC while longest pre-oviposition period (14.9±1.1 days) was observed at 24 ºC ($F_{(4, 49)}$ = 8.16, P= 0.0000). Number of egg cells produced in first brood differed significantly with the highest at 30 ºC (3.1±0.31) and the lowest at 24 ºC (1.9±0.23) (Fig. 1B) ($F_{(4, 49)}$ = 3.66, P= 0.0115). Number of workers in first brood was significantly higher at 28 ºC (7.9±0.45) which was insignificant at 26 ºC and 30 ºC (7.2+0.62 and 7.5+0.5). It was significantly lower at 24 ºC (5.6±0.30) ($F_{(4, 49)}$ = 3.81, P= 0.0094) (Fig. 1B).

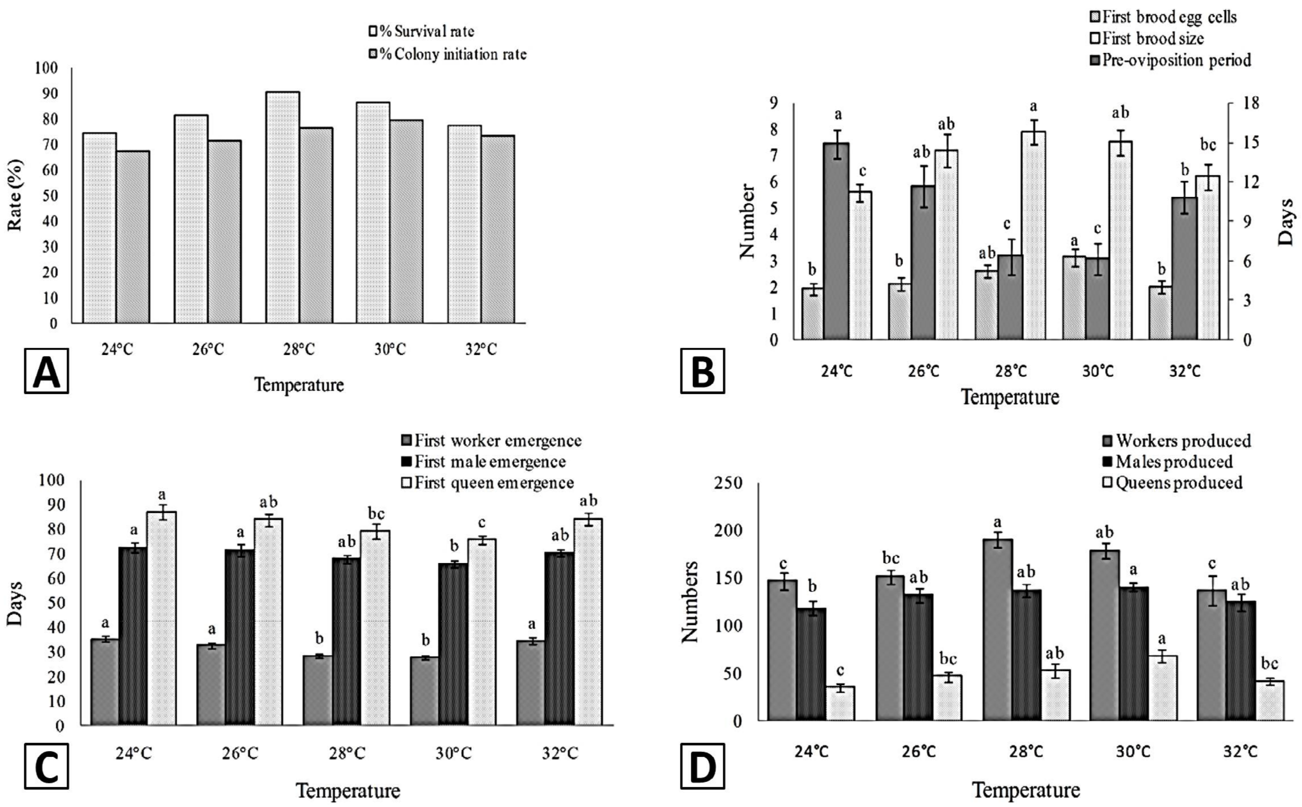

Fig. 1. Effect of different exposure temperatures on: survival and colony initiation rate of queens (A), pre-oviposition periods, production of egg cells and brood size in first batch (B), emergence timing of first worker, male and daughter queen (C), and total number of workers, males and progeny queens produced (D) for *Bombus terrestris*.

Table I.- Effect of temperature treatments on different characteristics of *B. terrestris* colony.

Parameters observed	Exposure temperature				
	24 ºC	26 ºC	28 ºC	30 ºC	32 ºC
Switch point (days)	37.7±2.07a	39.1±2.48a	39.5±1.91a	38.3±1.3a	36.2±1.7a
Competition point (days)	42.7±2.04a	43.8±2.42a	47.9±2.04a	48.6±2.65a	44.6±1.65a
Queen longevity (days)	98.8±3.55bc	105.2±4.18abc	110.8±5.43ab	113.7±6.15a	96.9±3.62c
Energy spent on sexual (KJ)	497.55±46.32	670.45±48.42	728.48±65.39	859.79±56.54	616.26±39.32

Data expressed as Mean± S.E. Means followed by different letter are significantly different (P≤0.05).

The emergence timing of first worker was observed shortest at 30 ºC (27.70±0.93 days) insignificant to 28 ºC (28.4±0.92) but significantly different at other three temperature treatments. It was 35.1±1.14 days at 24 ºC, 32.6±1.29 days at 26 ºC and 34.4± 1.19 days at 32 ºC ($F_{(4, 49)}$ = 9.48, P= 0.0000) (Fig. 1C). Insignificant difference in the emergence timing of males was observed. However, it was shortest at 30 ºC (66.00±1.22 days) and longest at 24 ºC (72.80±2.03 days) ($F_{(4, 49)}$ = 2.38, P= 0.0653) (Fig. 1C). Difference in emergence timing of daughter queens was significant with shortest at 30 ºC (75.80±1.82 days). It was statistically different to that at 24 ºC, 26 ºC and 32 ºC but differed insignificantly to that at 28 ºC. It took more time at 24 ºC (87.10±2.87 days) ($F_{(4, 49)}$ = 3.02, P= 0.0273) (Fig. 1C).

Total number of workers produced varied significantly by temperature exposures ($F_{(4, 49)}$ = 4.89, P= 0.0023) (Fig.1D). Highest number of workers were produced at 28 ºC (190±7.82), statistically similar to that at 30 ºC but significantly different from that at 24 ºC, 26 ºC and 32 ºC. Lowest number of workers was produced at 32 ºC (137±15.9). Production of males was insignificantly different however, the highest number (140±4.44) produced at 30 ºC and the lowest number (118±7.37) at 24 ºC ($F_{(4, 49)}$ = 1.79, P= 0.1480) (Fig. 1D). Production of daughter queens was significantly affected by temperature treatments with the highest (67.7±6.72) produced at 30 ºC. Lowest production of daughter queens (34.70±4.83) was observed at 24 ºC ($F_{(4, 49)}$ = 4.74, P= 0.0028) (Fig. 1D).

Table I shows different temperature on switch points, competition points, energy spent on sexuals and life span of founding queens of *B. terrestris*. There existed insignificant difference in occurrence of switch point at five different exposure temperatures ($F_{(4, 49)}$ = 0.45, P= 0.7713). It took 36-39 days to switch from worker to sexual production in colonies under observation at all temperatures. Insignificant difference was observed in the occurrence of competition point at five different temperatures ranging from 24-32 ºC. However, it occurred earlier at 24 ºC (42.70±2.04 days) and late at 30 ºC (48.60 ±2.65 days) ($F_{(4, 49)}$ = 1.40, P= 0.2491). Amount of energy spent on production of sexuals at different temperatures showed 860 KJ, spent on production of sexuals at 30 ºC, the highest among all the temperature treatments. The lowest was 498 KJ, spent at 24 ºC. There existed insignificant effect of different temperature treatments on lifespan of mother queen. However, it was the longest at 30 ºC (113±6.15 days) and 98.8±3.55 days at 24 ºC and shortest at 32 ºC (96.9±3.62 days) ($F_{(4, 49)}$ = 2.41, P= 0.634).

DISCUSSION

Among all the abiotic factors, temperature plays a vital role in growth and development of many beneficial insects especially B. *terrestris* reared on a commercial scale for pollination purpose (Obrycki and Tauber, 1981). Optimal development conditions are very much necessary to increase the efficiency of its mass rearing. In nature, after emergence from the diapause in spring, queen starts foraging flight for 10-15 days and search for a suitable place for initiation of its colony (Velthuis and van Doorn, 2006). The present study focused on observing the effect of five different exposure temperatures to colony initiation, growth and reproductive behavior of *B. terrestris* queens.

Results from our experiment showed that 28 ºC and 30 ºC temperatures showed the highest survival rate of queens after flight period of one week (90% and 86%, respectively). The highest percentage of the colony was initiated at 30 ºC. For early colony initiation and survival rate, 30 ºC was observed most suitable with 86% queen kept on egg laying (Gurel and Gosterit, 2008). Highest colony initiation (egg laid) and colony production ratio were also observed at the same temperature with 60% RH (Jie *et al*., 2005). However, there existed some variation for another species, *B. ignitus* queens with highest colony initiation percentage (83%) at 27 ºC with 65% RH (Yoon *et al*., 2002).

In the artificial rearing of *B. terrestris*, the most important stage after the diapause of queens is colony initiation (Velthuis and van Doorn, 2006). Queen with the shortest pre-oviposition period (colony initiation period) is considered very important having strong production abilities. We observed significantly shorter pre-oviposition period at 28 ºC and 30 ºC (6.30±1.35 and 6.10±1.15 days, respectively). However, the pre-oviposition period was found longer took more days (12.47±0.78 days) previously at the same temperature (Gurel and Gosterit, 2008). Another species, *B. ignitus*, however, took similar days (6.3±4.0 days) for pre-oviposition period at the same temperature (Yoon *et al*., 2002). This differed when increasing hibernation duration from three to four months with bit earlier (3.8±0.7 days) for *B. terrestris* queen at 28 °C (Amin *et al*., 2008) and variability existed (2.44±0.6 to 5.75±0.8 days) for field collected queens for pre-oviposition period which might be due to more severe field conditions and high stress for survival (Yeninar *et al*., 2000).

Temperature significantly affected the production of egg cells by bumblebee queens with highest egg cells produced (3.10±0.31) at 30 ºC. Production of egg cell in first brood with maximum numbers produced at 30 ºC for same species (Amin *et al*., 2008; Gurel and Gosterit, 2008). However, there existed little variation in cell numbers with previous work. Similarly, the ratio of egg laying and colony production was higher at 30±0.5 ºC and 60% relative humidity (Jie *et al*., 2005). Number of cells produced by field-collected queens was comparatively more than those of laboratory reared which might be due to genetic diversity, competition and other environmental factors to field collected queen's response (Yeninar and Kaftanoglu, 1997; Yeninar *et al*., 2000; Gosterit and Gurel, 2005).

Temperature also influenced activation and development of ovary in bumblebee queens with an increase of ambient temperature (Vogt *et al*., 1998; Amin *et al*., 2008). Similar response to this temperature has been observed for other insects like boll weevil, *Anthonomus grandis* (Boheman) suggesting 30 °C as most suitable for such reproductive mechanism but decreased significantly after that (Greenberg *et al*., 2005). Egg laying of *Adoxophyes honmai* (Lepidoptera: Tortricidae) significantly affected by temperature with the highest number of eggs laid between 28-30 °C (Nabeta *et al*., 2005). 30 °C influenced significantly the timing of first worker emergence with the shortest period (27.7±0.93 days) as previously observed for both *B. terrestris* and *B. ignitus*. However, the latter species performed well at 27 ºC with almost half duration for first worker emergence than former observed (Gurel and Gosterit, 2008; Yoon *et al*., 2002). Emergence timing of first brood workers play an important role in the development of the colony. Once the first brood worker emerges, they help the queen in the development of subsequent brood and share the workload of the queen.

Number of workers in first brood (7.9±0.45) were significantly more at 28 ºC which differ as variable temperatures showed no effect at three temperature regimes (24, 27 and 30 °C) (Gurel and Gosterit, 2008). However, there existed insignificant differences in emergence timing of males which were also observed previously for both *B. terrestris* and *B. ignitus* (Holland, 2013; Yoon *et al*., 2002). However, the shortest period of male emergence was at 27 ºC (71.3±5.1 days). Change of the founding queen pheromones and colony control play important role in the initiation of caste production in bumblebee colonies (Cnaani *et al*., 2000). In social Hymenoptera such as *A. mellifera* and *B. hypnorum*, juvenile hormone (JH) was found in the determination of castes (Röseler and Röseler, 1974; Rembold *et al*., 1974).

Emergence period of new queens were significantly affected by temperature with the shortest period at 30 ºC (75.80±1.82 days). Significant effect of temperature was, however, observed in queen emergence for *B. terrestris* (Gosterit and Gurel, 2005) taking slightly more time (89.7±6.33 days) than our study. Yoon *et al*. (2002), for *B. ignitus*, found the shortest period of queen emergence (80.1±4.5 days) at 27 °C. Such variation was observed for emergence timing of progeny queens in a colony in the response of temperature (Spark and Collinson, 2007; Bartomeus *et al*., 2011; Holland, 2013).

Switch and competition point are important stages in colony development of bumblebees, however, we observed insignificant effect of exposure temperatures to these stages in bumblebee biology. Gurel and Gosterit (2008) and Amin *et al*. (2008) also observed no effect of different rearing temperatures. These points might be endogenously controlled by the queen and insensitive to external temperature.

Exposure of founding queen to the different experimental temperature showed significant variation in the production of workers. Number of workers produced was higher at 28 °C (190.10±7.82) than other temperatures. However, multiple conditions vary such response as Amin *et al*. (2008) found that queens that had artificially hibernated for the duration of three months and given exposure temperature of 28°C; produced highest workers number (268±31.4) in the established colonies. Holland (2013) also found a significant effect of temperature on the number of workers produced in the colony. However, Gurel and Gosterit (2008) found insignificant effect of temperature on production of workers with a maximum at 24 °C (102.71±14.30). Yoon *et al*. (2002) when reared *B. ignitus* at 23, 27 and 30 °C found an insignificant effect on the production of workers, however, workers produced at 27 °C were greater than other temperatures under study (163.7±33.3). Insignificant difference existed for male production at different temperatures which strengthen our observations for *B. terrestris* has also observed for *B. ignitus* (Yoon *et al*., 2002; Gurel and Gosterit, 2008). However, exposure temperature of 36 °C was identified to produce a maximum number of males in a colony (Amin *et al*., 2008).

Founded queens exposed to 30 °C produced significantly higher number of progeny queens (67.70±6.72) which coincide with previous observations (Gosterit and Gurel, 2005; Holland, 2013). Amin *et al*. (2008) observed that queens hibernated for three months when reared under short-day conditions and exposed at 36 °C produced 119.3±16.8 queens. Our observations differ from that of Gurel and Gosterit (2008) and Yoon *et al*. (2002) who observed the insignificant effect of rearing temperature on the production of progeny queen in *B. terrestris* and *B. ignitus* colonies. We observed insignificant variation in the lifespan of the founding queen which has also been observed previously for both *B. terrestris* and *B. ignitus* species (Holland, 2013; Yoon *et al*., 2002).

CONCLUSIONS

The present study indicates highest survival and colony initiation rate at exposure temperature of 28 °C and 30 °C. The initial phase of colony growth, further colony development and reproductive potential of the colony was also observed best at 28-30 °C. We suggest temperature exposure of 28-30 °C for successful artificial breeding of *B. terrestris* that provide pollination service to greenhouse and tunnel farming system.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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