

## Original Article

# Reproductive biology of *Santolina ligustica* Arrigoni, a rare narrow-ranged species: implications for conservation

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### ABSTRACT

*Santolina ligustica* is a rare narrow-ranged species endemic to Liguria (Italy) for which a likely trend of decline has been recorded. For this reason, it would be important to assess the reproductive biology of this endemic species. In this study, we investigated the plant–pollinator interactions, the reproductive mode of the plant, and we evaluated both reproductive efficiency and seeds' germination performances. *Santolina ligustica* is an obligate cross-pollinating species with a generalist pollination system. The most frequent insects are Coleoptera belonging to the genus *Oedemera*. Seed set is low, but the seeds germinate under a wide range of temperatures. Taken together, our results indicate that *S. ligustica* is an insect pollinated and self-incompatible plant, in which low visitation rate, secondary pollen presentation and/or lack of compatible mate may cause a low seed set per capitulum. Nevertheless, the high number of capitula per plant and the high percentage of germination may assure high number of seedlings. Thus, the survival of *S. ligustica* is probably threatened by other factors such as fragmented distribution and habitat reduction because of land-use change. Under this scenario, we proved that species' decline is not directly related to reproductive failure and different studies are desirable to provide suggestions for the species conservation.

**Keywords:** Asteraceae; narrow-ranged species; pollen vectors; reproductive strategy; *Santolina ligustica* Arrigoni; seed germination

### INTRODUCTION

Many of the endemic plants in the Mediterranean Basin (nearly 40%) are restricted to a very small area (i.e. narrow-range species; [Thompson 2020](#)). Narrow-range species are usually characterized by particular habitat requirements and exhibit traits reflecting stress-tolerance ([Chapin et al. 1993](#), [Poorter and Garnier 1999](#)). Moreover, they often have low competitive ability ([Fiedler 1987](#), [Byers and Meagher 1997](#), [Imbert et al. 2012](#)) and they are also probably dispersal limited ([Essl et al. 2011](#)). Last, narrow-range species frequently present low genetic diversity at population level ([Karron 1997](#), [Gitzendanner and Soltis 2000](#)) because of genetic drift, reduced individual fitness ([Hedrick and Kalinoswki 2000](#)), and inbreeding depression ([Lavergne et al. 2004](#)). Therefore, narrow-range species are likely to have reduced adaptive capacity and high susceptibility to changes in their habitats. In the case of entomophilous species, several factors may affect the reproductive success, such as a limited number of available pollinators, competition with

surrounding flowering species, or a small population size, which may decrease the attractiveness to pollinators ([Sih and Baltus 1987](#), [Byers 1995](#), [Kunin 1997](#)). Reduced pollinator service might lead to a reduction of seed set and increase of extinction risk ([Bond 1994](#)). In this context, data collection on breeding system and reproductive output of narrow-range species is crucial to implement effective strategies for their conservation, as these factors influence long-term demographic pattern ([Eckert et al. 2010](#)). In fact, the population growth rate is mainly determined by the reproductive success, while patterns of genetic variability are determined by gene flow (through pollen and seeds; [Ellstrand 1992](#), [Hutchison and Templeton 1999](#)).

*Santolina ligustica* Arrigoni (Asteraceae) is an endemic species, restricted to very few localities in eastern Liguria (NW Italy). *Santolina ligustica* is a perennial shrub (up to 50–60 cm high), characterized by small capitula (4–6 mm diameter), with white disc florets ([Pignatti et al. 2017–2019](#)). The previous study of [Torricelli et al. \(1999\)](#) listed the species as 'critically

endangered' according to IUCN categories, based on the area of occupancy. The authors estimated an area of occupancy of  $\sim 4.5 \text{ km}^2$  and the presence of  $\sim 30\,000$  individuals, the species occurring mainly in garrigue and open habitats on ophiolitic substrate from the sea level up to 600 metres of altitude. [Torricelli et al. \(1999\)](#) suggested a likely trend of decline for the species, mainly because of competitions with other plants and shrubs, cultivation and pasture, and wood fires. Asteraceae, usually pollinated by generalist insects ([Fu et al. 2023](#)), are characterized by the secondary pollen presentation ([Funk et al. 2009](#)). This mechanism consists of the release of pollen grains in portions and its relocation from the anthers to the style, limiting the amount of pollen removed by the pollinators during a single visit, thus increasing the fitness of the plant ([Ladd 1994](#)). Nevertheless, the reproductive biology of this endemic species is still unknown.

In this study, we aimed at investigating the mode of reproduction and success of *S. ligustica*. To accomplish this objective, we: (i) evaluated the type of pollinator and the frequency of visits; (ii) quantified the reproductive output of the plants; and (iii) evaluated the seeds' germination performances.

## MATERIAL AND METHODS

### Capitula visitors

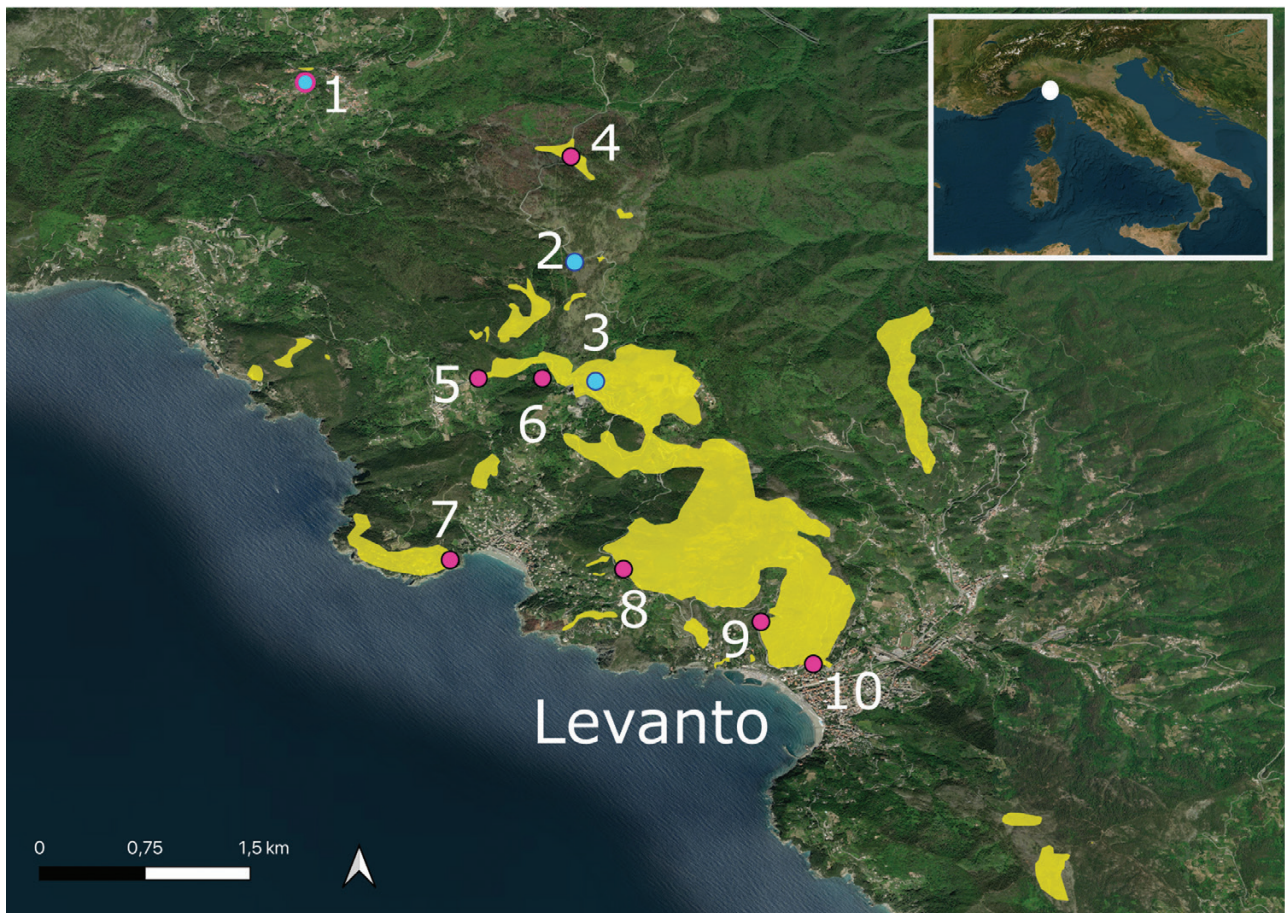
In July 2021, we documented insect visits and activity on *S. ligustica* inflorescences at the peak of the flowering season in

one population (pop 1, see [Fig. 1](#)). From two to four observers simultaneously monitored insect presence for periods of 15 minutes from 7 a.m. to 9 p.m. for a total time of 1155 minutes. We marked four areas of observation comprising about 100 capitula in an area of  $15 \times 15 \text{ cm}$ , each observer recorded all visitors that made contact with the capitula. Insects were identified directly in the field or collected for laboratory identification. We calculated the visitation rate for each capitulum by dividing the total visits observed in each time slot by the number of capitula observed and by the total observation time of that time slot. The visitation rate was calculated with R software ([R Development Core Team 2011](#)).

### Mode of reproduction

The mean number of florets per capitulum was calculated from 68 capitula (the ones used to investigate the breeding system, see next). We calculated the florets as mature fruits plus aborted seeds plus unfertilized ovules. The presence of hermaphrodite, female, and male florets was checked on three randomly selected capitula under a Leica M205 C stereomicroscope equipped with Leica EC3 digital colour.

In Asteraceae, physical isolation of individual florets is very difficult, given their small size and proximity in the capitula. Self-fertility is consequently measured by excluding pollinators from the entire capitulum, a procedure that does not allow for discarding the possibility of accidental geitonogamy ([Ueckermann and van Rooyen 2000](#)).



**Figure 1.** Distribution of the 10 populations of *S. ligustica* investigated in the present study.

During summer 2021, we studied the breeding system of *S. ligustica* in three populations (pop 1, pop 2, and pop 3, see Fig. 1). In total, we randomly chose 34 individuals at a distance of at least 1 m from each other: for each individual, we bagged one capitulum with non-woven fabric to test self-fertility and we randomly chose one capitulum exposed to open pollination as control. A total of 68 mature capitula from the two treatments were harvested in August and the numbers of fruits (cypselae) produced per capitulum were counted. Cypselae were all visually evaluated to estimate their quality (well-formed vs. empty) and counted under a Leica M205 C stereomicroscope. Mean percentage values and standard deviations were calculated with R software (R Development Core Team 2011).

### Seed germination

Seed germination was used as proxy of progeny performance, and it was evaluated in temperature- and light-controlled incubators. We collected seeds from nine populations (pop 1, 3, 4–10, see Fig. 1), the seeds were subjected to visual inspection and only filled seeds were used in the germination experiments. We tested eight temperatures from 10 to 27.5°C, at 2.5°C intervals, with a photoperiod of 12/12 light/dark. In each test, for each population we sowed 100 seeds in four Petri dishes (25 seeds per dish) containing two moist discs of filter paper. Germination was checked every 2 days for 32 days, and seeds were considered germinated when radicle emergence was > 1 mm. We estimated the percentage of germination at day 32 and  $T_{50}$  (i.e. number of days required for half the seeds to germinate) using the function 't50' of the library 'germinationmetrics' (Aravind *et al.* 2022) implemented in R.  $T_{50}$  was used as a proxy for seed germination speed (lower  $T_{50}$  values indicate faster germination).

## RESULTS

### Capitula visitors

A total of 37 different taxa of insect were observed (Supporting Information, Table S1). The most abundant visitors were Coleoptera (72.7%) followed by Hymenoptera (21.8%); all other insect taxa were included in the remaining 5.5% (Table 1). Among Coleoptera, Oedemeridae (46.4%) was the most frequent family, in particular the species *Oedemera flavipes* (42.45%), while among Hymenoptera, Formicidae (16.4%) was the most frequent family (Supporting Information, Table S1).

**Table 1.** List and abundances of insect visitor taxa observed on *Santolina ligustica* capitula.

Order	Percentage (%)
Blattaria	0.18
Coleoptera	72.73
Diptera	2.84
Hymenoptera	21.77
Lepidoptera	1.37
Neuroptera	0.09
Orthoptera	0.18
Rhynchota	0.82
	100

The visitation rate ranged from 0.0031 visit per minute per capitulum (time slot 7–8 a.m.) to 0.0139 visit per minute per capitulum (time slot 12–1 p.m.). The highest values (visitation rate > 0.01) were observed from 10 a.m. to 4 p.m. (Fig. 2, Supporting Information, Table S2).

### Mode of reproduction

Each capitulum bore a mean number of 70.78 (SD 18.02) florets. The anthesis of the capitulum, spirally developed from the periphery to the centre. All the florets of the analysed capitula were hermaphrodite. Overall, in the 34 open-pollinated capitula, the 8.06% (SD 12.06) of the florets produced well-formed seeds, whereas none of the 34 bagged capitula produced fruits (Supporting Information, Table S3). The number of capitula per plant can range from a dozen to hundreds.

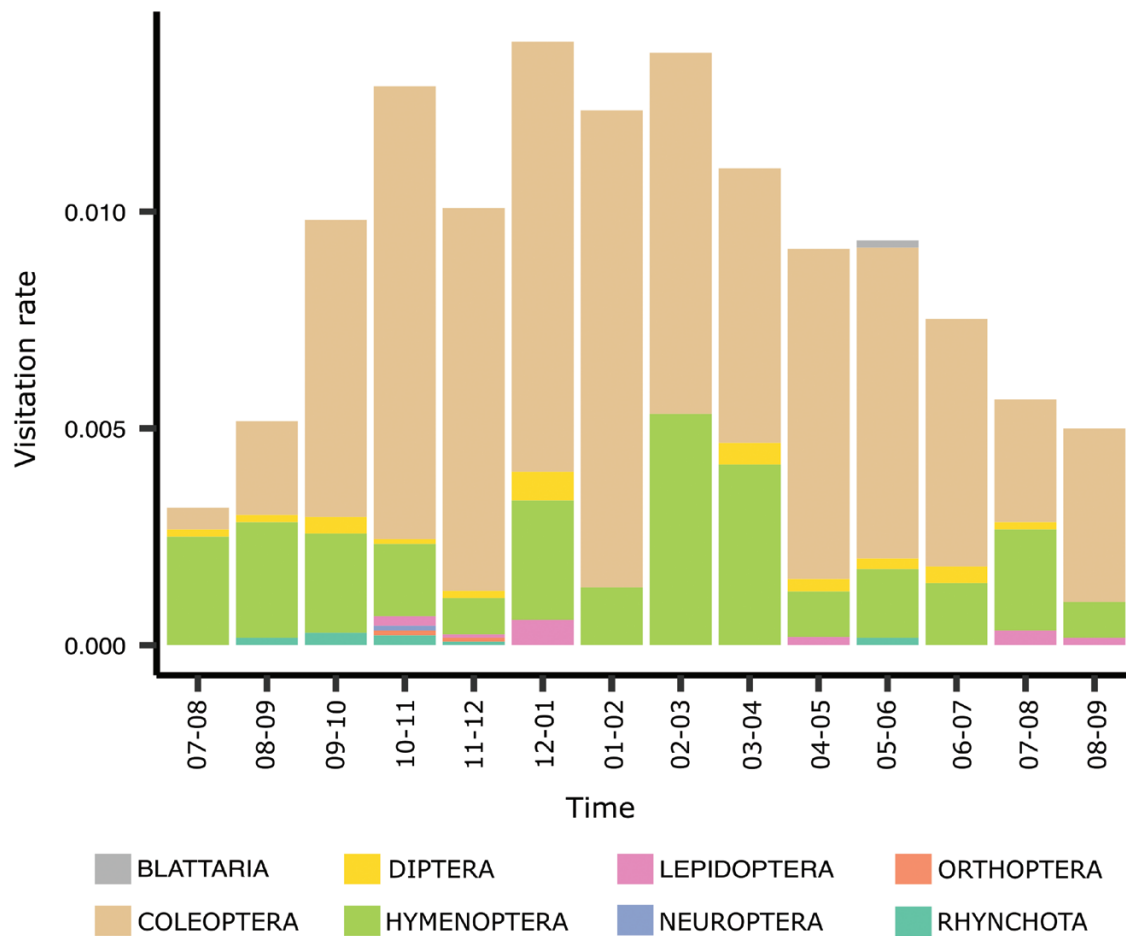
### Seed germination

The proportion of germinated seeds was high, ranging from 53.1% to 89.9% in all tested temperatures, the highest values were recorded in temperature ranging from 15 to 22.5°C (Fig. 3). Similarly, the germination was faster for temperatures between 17.5 and 22.5°C, while it slowed down in the warmest temperatures (25 and 27.5°C; Fig. 3) and far more in the coldest conditions (10 and 12.5°C; Fig. 3).

## DISCUSSION

In *S. ligustica* the most abundant visitors (Table 1) were Coleoptera (72.7%) and Hymenoptera (21.8%), suggesting a generalist pollination system. Most of the observed Coleoptera belongs to the genus *Oedemera* (Supporting Information, Tables S1 and S2). *Oedemera* species are known as obligate pollen feeders (Atanassova and Sivilov 2014) that prefer various species of Asteraceae as pollen source (Carloni and Bologna 2005, Sivilov *et al.* 2011, Atanassova and Sivilov 2014). In particular, small white flowers with pollen grains outside the corolla tube, as *Santolina* flowers, are known for usually attracting *Oedemera* species characterized by short proboscis (0.9 mm; Kugler 1984). Our data showed that the visitation rate observed in *S. ligustica* (Fig. 2) is lower in comparison to other studies on Mediterranean plants (Ne'eman *et al.* 2000, Potts *et al.* 2001, Tobajas *et al.* 2023). Both Formicidae and Oedemeridae are characterized by poor mobility, and they usually may spend longer time on the same capitula and visiting less capitula than bees (Bosch 1992), thus Formicidae and Oedemeridae might perform poor cross-pollination.

The lack of fruits in bagged capitula suggests that *S. ligustica* is a self-incompatible species and that it depends on pollinators for its reproductive success (Supporting Information, Table S2). Consequently, the low reproductive output recorded (i.e. seed set 8.06%) may be explained by the low visitation rates and by the low pollination efficiency of the main visitors. However, some studies suggest that a low visitation rate does not necessarily turn out in a low reproductive success (Hegland and Totland 2012, Lundgren *et al.* 2013). Thus, another possible explanation for the low seed set observed relies on the availability of compatible mating types, fundamental for the reproductive success of self-incompatible plant species. In small

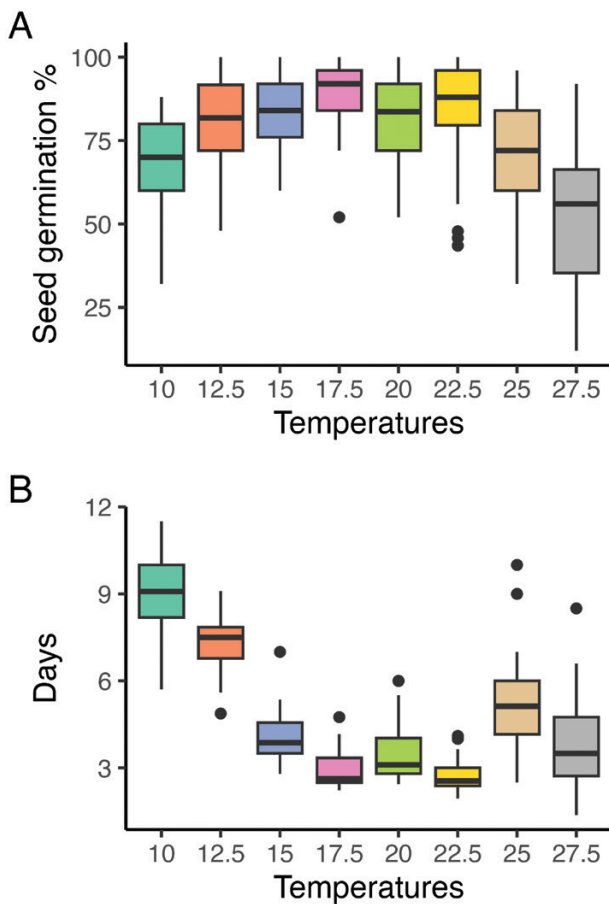


**Figure 2.** Visitation rate (mean number of visitors per minutes of observation per capitulum) on *S. ligustica* from 7 a.m. to 9 p.m.

populations, genetic drift can cause mating types to be biased towards close relatives or be lost altogether (Wright 1969, Les *et al.* 1991, Campbell and Husband 2007). Therefore, most pollination may result inefficient because of the dominance by one or a few genetically too similar mating types in the pollen transferred in adequate or even excessive amounts (Ågren 1996, Waites and Ågren 2004). Last, a low seed set might be a consequence of the self-interference i.e. a conflict between male and female functions (Webb and Lloyd 1986, Ladd 1994, Barrett 2002), because of the secondary pollen presentation. Secondary pollen presentation (common to all Asteraceae) is a mechanism for improving the efficiency and accuracy of pollen transfer and/or reception, thereby increasing the fitness of the plants (Lloyd and Yates 1982, Ladd 1994). However, in self-incompatible species, the proximity of stigmas and anthers might result in self-interference, with negative effects on plant fitness (Cesaro *et al.* 2004, Kawagoe and Suzuki 2005, Waites and Ågren 2006, Castro *et al.* 2008). In self-incompatible Asteraceae, the deposition of self-pollen, that cannot germinate on stigmas of the same plant, can cause stigma clogging (Barrett 2002), thus reducing access of pollen from other individuals to stigma surfaces and the probability to set seeds (Barrett 2002, Love *et al.* 2016). Moreover, also the predation of fruits by a parasite belonging to the Tephritidae (personal observation)

may result in a reduction of seeds production. Tephritidae are known for being one of the dominant pre-dispersal predators of Asteraceae (Zhang *et al.* 2018). Usually female of Tephritidae penetrate developing capitula and oviposit eggs into the capitula, where the eggs hatch into larvae that grow consuming developing seeds (Xi *et al.* 2015).

Seeds of *S. ligustica* can germinate immediately after harvesting, this result suggests that seeds are non-dormant. Moreover, the *S. ligustica* seeds germinate under a wide range of temperatures (Fig. 3), with the optimal temperatures  $\sim 17.5\text{--}22.5^\circ\text{C}$ . This result is in line with previous findings in Mediterranean environments where the germination is favoured at relatively low temperatures (between 15 and  $22^\circ\text{C}$ ) (Luna *et al.* 2012). These results indicate that *S. ligustica* germinates in the early autumn, a behaviour typical of species occurring in habitats with dry summers (Carta *et al.* 2013). These species usually germinate readily when water availability, soil moisture, and rainfall are high, and temperatures are not excessively prohibitive for seedlings establishment (Picciau *et al.* 2019). The high final germination proportions in all tested temperatures together with the low reproductive output per capitulum is consistent with the theory of inbreeding depression, according to which in outcrossing species inbreeding depression acts mainly at the late life stage, such as during seed production (Husband and Schemske 1996).



**Figure 3.** A, Germination performance and B,  $T_{50}$  at the different tested temperatures.

The suggested inbreeding depression, in addition to other factors, such as the decrease in autumn rainfall and the increase in competition with other plants and shrubs, might cause the species decline observed by [Torricelli \*et al.\* \(1999\)](#).

### CONCLUSION

The present study revealed that *S. ligustica*, an Italian rare range-restricted species, is an insect pollinated and self-incompatible plant in which low visitation rate, secondary pollen presentation, and/or lack of a compatible mate may cause a low seed set per capitulum. Nevertheless, it is important to highlight that plants can bear dozens to hundreds of capitula, producing a high number of seeds per plant. Thus, the survival of *S. ligustica* may be threatened by other factors such as fragmented distribution and habitat reduction because of land-use change ([Torricelli \*et al.\* 1999](#)). In fact, self-incompatible species are expected to be more susceptible to habitat fragmentation ([Aguilar \*et al.\* 2006](#)). In species dependent on animal pollinators for successful sexual reproduction, habitat fragmentation may modify richness, composition, abundance, and/or behaviour of pollinators or the availability of conspecific mates, thus making seed production vulnerable ([Aguilar \*et al.\* 2006](#)). Moreover, the wide range of temperatures under which germination occurs suggests that *S. ligustica* is little dependent on temperatures, thus water supply may be the main determinant of the timing of germination. Thus, the increase in aridity forecasted in the Mediterranean region ([Cramer \*et al.\* 2018](#)) will represent another future threat to species persistence.

### SUPPLEMENTARY DATA

Supplementary data is available at *Botanical Journal of the Linnean Society* online.

### ACKNOWLEDGEMENTS

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### CONFLICT OF INTEREST

None declared.

### DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material.

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