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ORIGINAL ARTICLE

Cytogenetics, geographical distribution, pollen stainability and fecundity of five diploid taxa of *Santolina rosmarinifolia* L. aggregate (Asteraceae: Anthemideae)

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Abstract The chromosome analysis of *Santolina rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens* and the hybrid complex (*S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids) shows that all the taxa are diploids ($2n = 2x = 18$; 18 + 1 or more B chromosomes, with $2n = 19$, 20 only in the hybrid complex). The results show a conserved general structure of the karyotype (14m + 2sm + 2st), but in *S. semidentata* subsp. *melidensis* it is variable, with 14m + 2sm + 2st in ten individuals, 14m + (1m − 1sm) + (1 m − 1st) in nine individuals and 12m + (1m − 1sm) + (1m − 1st) + 2st + 1B in five individuals. Tetraploid individuals occurred in the diploid populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. canescens*, and their autopolyploid origin is discussed. Multivalent configurations at diakinesis, simple and double chromosome bridges and delayed disjunction of homologous and non-homologous chromosomes at anaphase I have negative effects on pollen stainability. The mean fructification percentage is moderate. The results suggest that the complex is a mosaic of introgressive hybrids.

Keywords Diploid · Hybridisation · Nested MANOVA · Multiple regression · Pollen stainability · Fecundity · *Santolina*

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Introduction

Hybridisation and the maintenance of hybrid zones have received considerable attention traditionally because of their important contribution to the understanding of evolutionary patterns and processes (Barton and Hewitt 1989; Rieseberg 1997; Rieseberg et al. 2007; Barber et al. 2007). Hybridisation—the production of viable offspring from interspecific matings—occurs in roughly 10% of animal species and 25% of plant species (Larson 1968 cited by Baack and Rieseberg 2007). Studies of hybrid zones provide insights into evolutionary and speciation processes (Barton and Hewitt 1981; Harrison 1990; Rieseberg 1995; Arnold 1997; Allendorf et al. 2001). In the simplest case, the parental races differ by a single chromosomal rearrangement or even by only a single-gene difference (P.E. Brandham, Royal Botanical Garden, Kew, personal communication). The hybrid zone between diploid genetic entities have been the subject of several works (e.g. Arnold 1997; Rieseberg 1997) and are often important for evolutionary change because hybridisation and introgression can (1) increase genetic diversity within species, (2) cause the transfer of genetic adaptations, (3) induce the breakdown of reproductive barriers between closely related groups, or conversely reinforce them, and (4) lead to the emergence of a new ecotype or species (Barton and Hewitt 1989; Abbott 1992; Rieseberg 1997; Cozzolino et al. 2006).

Ellstrand et al. (1996) found that *Asteraceae* is one of the most important families in which intra-generic hybridisation occurs. The *S. rosmarinifolia* aggregate comprises nine taxa, principally diploids (six taxa). One of them (*S. pectinata* [Rivero-Guerra, 2008a]) has two cytotypes: diploid and tetraploid, and the other two are tetraploid (*S. rosmarinifolia* subsp. *arrabidensis* [Rivero-Guerra, 2008b]) and hexaploid (*S. ageratifolia* [Rivero-Guerra, 2008c]), respectively.

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Santolina rosmarinifolia L. subsp. *rosmarinifolia* is sympatric with *S. oblongifolia* Boiss., an endemic of the Iberian Central Systems, where spontaneous hybridisation and introgression occur. As a consequence, several botanists have granted taxonomic status to the intra-populational variation of the populations of the Central Iberian System, for example, Willkomm and Cutanda, in Willkomm (1859) (*S. heterophylla*), Willkomm, in Willkomm and Lange (1870) (*S. oblongifolia* var. α. *obtusifolia* and *S. oblongifolia* var. β. *ceratophylla*), Jordan and Fourreau (1869) (*S. lobata* and *S. sericea*) and Guinea (1970) (*S. rosmarinifolia* var. *lobata*). Furthermore, Valdés-Bermejo and López (1977) suggested for the first time that *S. canescens* (with southern distribution on calcareous soils) may have arisen by hybridisation between *S. rosmarinifolia* and *S. pectinata*, but they did not provide any meiotic data supporting their proposal.

The analysis of chromosome pairing behaviour and meiotic abnormalities provided some indications concerning the hybridisation and helped to evaluate the impact of hybridisation on plant speciation and the degree of differentiation between hybridising taxa (Rieseberg et al. 2000). Valdés-Bermejo et al. (1981) published chromosome counts for *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata*, *S. canescens*, and *S. oblongifolia*; while Rodriguez-Oubiña and Ortiz (1993) published the only known chromosome count for *S. semidentata* subsp. *melidensis*. No detailed analyses have been published regarding karyotype variation, meiotic configurations, chiasma frequency, pollen stainability or fecundity in *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens*, *S. oblongifolia*, nor in the hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (referred to as 'hybrid complex' in the text). The first aim of this work was to analyse the variation in the above-mentioned parameters within and between taxa and within the hybrid complex, as well as the geographical distribution and ecology of the taxa.

A study of the cytogenetics of 18 populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (Rivero-Guerra 2008b) in the Iberian Peninsula shows the existence of two ploidy levels: diploid and tetraploid. Diploid cytotypes of *S. rosmarinifolia* subsp. *rosmarinifolia* are located in the Central Iberian Peninsula, running northwards in the Peninsula, in the western and Central Iberian Systems, whereas tetraploid cytotypes (*S. rosmarinifolia* subsp. *arrabidensis*) are located in Serra da Arrábida, Portugal. Both show regular meiosis. Furthermore, the populations of central Spain can scarcely be differentiated from *S. rosmarinifolia* subsp. *rosmarinifolia* (Rivero-Guerra 2009b). The second aim of this work was to add to the above finding by Rivero-Guerra (2008b) in the biology of *S. rosmarinifolia* subsp. *rosmarinifolia*.

The general hyphothesis of this work is based on the facts that these taxa show a tendency to preserve the general structure of their karyotypes and this does not preclude the presence of paracentric inversions. In addition, despite the high degree of sympatry and a conservative tendency towards maintaining the general structure of the karyotype in the *S. rosmarinifolia* aggregate's diploid taxa, this does not support a genetic barrier between them, so that hybridisation and backcrossing or "introgressive hybridisation" occur.

The study investigates the following questions: Do *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids show somatic chromosome variation within the hybrid complex? Do *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia* exhibit analogous meiotic behaviour, pollen stainability and fecundity within and outside the hybrid complex?

Materials and methods

Sampling

The study covered 28 populations of *S. rosmarinifolia* subsp *rosmarinifolia* (334 individuals), 5 of *S. oblongifolia* (95 individuals), 10 of *S. semidentata* subsp. *semidentata* (140 individuals), 1 of *S. semidentata* subsp. *melidensis* (39 individuals), 25 of *S. canescens* (222 individuals) and 9 of the hybrid complex, where *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids grow together or one parent grows together with the presumed hybrids (261 individuals). These are detailed in Table 1. The populations were sampled in the summer of 1997.

Cytogenetics and pollen stainability

The study of somatic chromosomes was carried out on root-tip meristems obtained from germinating achenes, the latter originating from natural populations (Table 1). The root tips were treated with eight-hydroxyquinoline (0.002 M) (Tjio and Levan 1950) and fixed in Farmer's fluid [one part glacial acetic acid and three parts ethanol (95–100%)] (Löve and Löve 1975). In the study of meiosis and pollen stainability, flower buds were fixed in the field (Table 1) in Carnoy's fluid [one part glacial acetic acid, three parts chloroform and six parts ethanol (95–100%)] [Löve and Löve 1975]. The root tips and the anthers were stained with alcoholic hydrochloric acid-carmine (Snow 1963) and were squashed on slides in 45% acetic acid. Chromosome number and karyotype morphology (length of the long and short arm, total length of the chromosome excluding the satellite) and the chromosome ratio (length of the long arm/length of the short arm), the number of satellites, the karyotype formula (according to the terminology of Levan et al. 1964),

Table 1 Location of the studied populations of *S. oblongifolia* (OBL), *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN), and of the hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB). The chromosome number and the number of individuals studied per population are shown

Pop.	Location	Taxon	2*n* (*N*)	*N*				
				KF	KM	M	PS	F
1	Álava, Lantaron, Fontecha, 42°44′30″N 30°01′04″W, 464 m, conglomerates and limestone	ROS	18 (23)	15	15	8	8	8
2	Álava Bóveda, 42°37′78″N 7°29′03″W, 369 m, marl-limestone, marl, clay, and limestone	ROS	18 (10)	–	–	10	10	10
3	Ávila: between El Barco de Ávila and Puerto del Pico, at San Bartolomé de Tormes crossroads, 40°21′26″N 5°16′47″W, 1,350 m, granite	ROS	18 (15), 18 + 1B (2)	15	15	–	–	–
4	Ávila: Hoyos del Espino, 40°21′39″N 5°10′13″W, 1,498 m, granite	ROS	18 (15), 18 + 1B (2)	5	–	10	10	10
5	Ávila: Santiago de Aravalle, 40°18′90″N 5°37′36″W, 1,195 m, granite	ROS	18 (5)	5	–	–	–	–
6	Ávila: between El Barco de Ávila and Puerto del Pico, 17 Km from El Barco de Ávila, 40°21′75″N 5°32′35″ W, 1,019 m, granite	ROS	18 (15), 18 + 1B (2)	5	–	–	–	10
7	Ávila: Hoyos del Collado, 40°21′55″N 5°12′33″W, 1,545 m, granite	ROS	18 (15), 18 + 1B (1)	15	15	–	–	–
8	Ávila: Navarredonda de Gredos, 40°21′23″N 5°09″54″W, 1,550 m, granite	ROS	18 (12)	5	–	–	–	7
9	Ávila: Venta de Raquilla, 40°22′74″N 5°01′82″W, 1,238 m, granite	ROS	18 (15), 18 + 1B (2)	15	15	–	–	–
10	Ávila: Barco de Ávila, 40°22′25″N 5°31′31″W, 1,021 m, granite	ROS	18 (14), 18 + 1B (2)	5	–	9	9	9
11	Ávila: Cuevas del Valle, 40°17′59″N 5°00′38″W, 843 m, granite	ROS	18 (15), 18 + 1B (5)	15	15	–	–	–
12	Ávila: Garganta de los Caballeros, 40°16′97″N 5°32′22″W, 1,519 m, granite	ROS	18 (15), 18 + 1B (4)	5	–	10	10	10
13	Ávila: La Aliseda de Tormes, 40°19′53″N 5°24′12″W, 1,136 m, granite	ROS	18 (5), 18 + 1B (2)	5	–	–	–	–
14	Burgos, Fresneda de la Sierra, 40°23′10″N 2°08′54″W, 966 m, slate, limestone and quartzite	ROS	18 (10)	–	–	10	10	10
15	Madrid, Sierra de Guadarrama, Miraflores de la Sierra, 40°48′51″N 3°44′05″W, 1,034 m, slate and grauwackes	ROS	18 (10)	–	–	10	10	10
16	Salamanca: Castellanos de Villiquera, 41°02′65″N 5°40′52″W, 800 m, granite	ROS	18 (15), 18 + 1B (2)	5	–	10	10	10
17	Salamanca: Calzada de Valdunciel, 41°04′67″N 5°41′62″W, 807 m, granite	ROS	18 (15), 18 + 1B (5)	15	15	–	–	–
18	Salamanca: Vallejera de Riofrío, 40°24′62″N 5°43′77″W, 1,115 m, granite and sand	ROS	18 (5), 18 + 1B (2)	5	–	–	–	–
19	Salamanca: Béjar, 40°22′22″N 5°44′51″W, 1,022 m, granite	ROS	18 (15), 18 + 1B (7)	15	15	–	–	–
20	Segovia, San Rafael, 40°42′37″N 4°10′16″W, 1,321 m, granite	ROS	18 (10)	–	–	–	10	10
21	Toledo: Puebla de Montalbán, 39°50′65″N 4°23′81″W, 420 m, sand, clay and limestone	ROS	18 (5), 18 + 1B (2)	5	–	–	–	–
22	Toledo: Mocejón, 39°56′34″N 3°54′29″W, 475 m, sand, clay, gypsum and limestone	ROS	18 (23); 36 (2) 18 + 1B (2)	15	15	10	10	10
23	Toledo: Azucaica, 39°52′87″N 3°59′33″W, 458 m, limestone, clay and sandstone	ROS	18 (15), 18 + 1B (2)	15	15	–	–	–
24	Toledo: between Talavera de la Reina and Calera y Chozas, 39°55′20″N 4°54′55″W, 363 m, conglomerates, sand, sandstone, limes and clay	ROS	18 (5), 18 + 1B (1)	5	–	–	–	–
25	Toledo: San Pablo de los Montes, Las Navillas, 39°33′20″N 4°21′86″W, 864 m, granites	ROS	18 (5)	5	–	–	–	–
26	Zamora: Corrales, 41°20′08″N 5°43′13″W, 767 m, marl, marl-limestone, limestone and quartzite	ROS	18 (5), 18 + 1B (1)	5	–	–	–	–
27	Zamora: Ferreras de Abajo, 41°54′08″N 6°03′65″W, 792 m, marl, marl-limestone, limestone and quartzite	ROS	18 (15), 18 + 1B (1)	15	15	–	–	–

Table 1 continued

Pop.	Location	Taxon	2*n* (*N*)	*N*				
				KF	KM	M	PS	F
28	Zamora: Ferreras de Arriba, 41°53′39″N 6°10′67″W, 899 m, marl, marl-limestone, limestone and quartzite	ROS	18 (5), 18 + 1B (1)	5	–	–	–	–
29	Ávila: Navalguijo, 40°15′68″N 5°31′93″W, 1,204 m, granite	OBL	18 (15)	15	15	–	–	–
30	Ávila: La Mira, 40°15′59″N 5°10′28″W, 2,200 m, granite	OBL	18 (25)	15	15	10	10	10
31	Ávila: Canchal Negro, 40°20′88″N 5°40′27″W, 2,000 m, granite	OBL	18 (25)	15	15	10	10	10
32	Salamanca: Béjar, La Garganta, 40°19′48″N 5°49′10″W, 1,000 m, granite	OBL	18 (15)	15	15	–	–	–
33	Salamanca: Calvitero, 40°17′16″N 5°44′18″W, 2,360 m, granite	OBL	18 (15)	5	–	10	10	10
34	León: Km 43 on the road from Villar del Monte to Nogareja, 870 m, slate and quartzite	SEM	18 (15)	15	15	–	–	–
35	León: ascending to Peña Trevinca from La Baña, 42°15′03″N 6°43′65″W, 1,520 m, slate and quartzite	SEM	18 (15)	5	–	10	10	10
36	León: ascending to El Mirador de Las Médulas from Carrucedo, 42°28′92″N6°45′50″W, 630 m, slate and quartzite	SEM	18 (15)	15	15	–	–	–
37	León: Las Médulas, 42°27′20″N 6°45′74″W, 818 m, alluvial	SEM	18 (15)	15	15	–	–	–
38	León: ascending to El Morrederos from Corporales, 42°23′94″N 6°30′68″W, 1,834 m, slate and quartzite	SEM	18 (25)	15	15	10	10	10
39	León: Montes de Valdueza, 42°26′40″N 6°35′77″W, 1,155 m, slate and quartzite	SEM	18 (15)	15	15	–	–	–
40	León: Ambasaguas, 42°43′28″N 5°22′72″W, 994 m, slate and quartzite	SEM	18 (25)	15	15	10	10	10
41	Zamora: San Ciprian, 42°10′69″N 6°39′27″W, 1,241 m, slate and quartzite	SEM	18 (5)	5	–	–	–	–
42	Zamora: Ribadelago, 42°07′06″N 6°44′19″W, 1,008 m, slate	SEM	18 (5)	5	–	–	–	–
43	Zamora: Ríonegro del Puente, 42°00′92″N 6°19′65″W, 860 m, slate and quartzite	SEM	18 (5)	5	–	–	–	–
44	La Coruña: Santiso, area of Barazón, 42°52′49″N 8°04′24″W, 410 m, serpentine	MEL	18 (39)	24	24	15	15	15
45	Almería: Esfiliana, 37°13′35″N 3°05′45″W, 1,085 m	CAN	18 (15), 36 (2)	5	–	12	12	12
46	Almería: Sierra de los Filabres, ascending to Calar Alto from Gergal, 37°13′05″N 2°33′18″W, 1,900 m, schist and quartzite	CAN	18 (5)	5	–	–	–	–
47	Almería: Sierra de los Filabres, Calar Alto, 37°13′12″N 2°32′01″W, 2,000 m, schist and quartzite	CAN	18 (15)	15	15	–	–	–
48	Almería: Sierra de los Filabres, Bacares 37°15′73″N 2°27′11″W, 1,257 m, schist and quartzite	CAN	18 (5)	5	–	–	–	–
49	Almería: Sierra de los Filabres, Tijola, 37°21′55″N 2°26′78″W, 656 m, schist and quartzite	CAN	18 (5)	5	–	–	–	–
50	Cádiz: Sierra de Grazalema, between Puerto de Las Palomas and Grazalema, 36°47′88″N 5°22′21″W, 1,091 m, limestone	CAN	18 (15)	15	15	–	–	–
51	Córdoba: Sierra Horconera, Rute, 37°20′68″N 4°21′91″ W, 796 m, marl–limestone	CAN	18 (5)	5	–	–	–	–
52	Jaén: Fuensanta de Martos, 37°39′37″N 3°53′68″W, 876 m, mal	CAN	18 (26); 36 (4)	15	15	15	15	15
53	Jaén: Torre del Campo, 37°46′00″N 3°52′00″W, 673 m, marl	CAN	18 (5)	5	–	–	–	–
54	Jaén: Jabalcuz, 37°43′24″N 3°48′19″W, 759 m, marl	CAN	18 (5)	5	–	–	–	–
55	Jaén: Los Villares, 37°42′94″N 3°48′96″W, 612 m, marl	CAN	18 (15)	15	15	–	–	–
56	Jaén: Valdepeñas de Jaén, 37°36′63″N 3°48′00″W, 950 m, marl	CAN	18 (5)	5	–	–	–	–
57	Jaén: Alcalá la Real, 37°28′96″N 3°51′77″W, 909 m, marl	CAN	18 (5)	5	–	–	–	–
58	Jaén: Fraile, 37°29′44″N 3°48′95″W, 1,050 m, marl	CAN	18 (5)	5	–	–	–	–
59	Granada: Zafarraya, 36°59′01″N 4°10′94″W, 920 m, limestone	CAN	18 (5)	5	–	–	–	–

Table 1 continued

Pop.	Location	Taxon	2*n* (*N*)	*N*				
				KF	KM	M	PS	F
60	Granada: Puerto de la Mora, 37°15′00″N 3°28′01″W, 1,347 m, limestone	CAN	18 (5)	5	–	–	–	–
61	Granada: Pinos Genil, 37°09′60″N 3°31′23″W, 780 m, schist	CAN	18 (5)	5	–	–	–	–
62	Granada: Baza, 37°30′26″N 2°46′83″W, 828 m, conglomerates	CAN	18 (5)	5	–	–	–	–
63	Granada: Galera, 37°44′53″N 2°32′74″W, 838 m, conglomerates	CAN	18 (15)	15	15	–	–	–
64	Granada: Sierra Nevada, between Dornajo and Hotel del Duque, 37°08′33″N 3°25′96″W, 1,473 m, schist	CAN	18 (5)	5	–	–	–	–
65	Granada: Sierra Nevada, between Bérchules and Puerto de la Ragua, 36°59′47″N 3°08′68″W, 1,316 m, schist	CAN	18 (5)	5	–	–	–	–
66	Málaga: Antequera, El Torcal, 36°59′08″N 4°33′77″W, 622 m, limestone	CAN	18 (5)	5	–	–	–	–
67	Málaga: Antequera, Sierra de Cabra, 37°30′84″N 4°24′83″W, 584 m, limestone	CAN	18 (15)	15	15	–	–	–
68	Sevilla: between El Saucejo and Osuna, 37°06′25″N 5°06′82″W, 551 m, limestone	CAN	18 (5)	5	–	–	–	–
69	Sevilla: Sierra del Tablón, between Pruna and Algámitas, 37°00′85″N 5°10′00″W, 722 m, limestone	CAN	18 (15)	15	15	–	–	–
70[a]	Ávila: near to Plataforma de Gredos, 40°17′72″N 5°13′62″W, 1,665 m, granite	ROS, OBL, HYB	18 (34), 18 + 1B (9)	15	15	19	19	19
71[a]	Ávila: Puerto del Tremedal, 40°22′90″N 5°37′09″W, 1,602 m, granite	ROS, OBL, HYB	18 (15), 18 + 1B (3)	15	15	–	–	–
72[a]	Ávila: San Bartolomé de Béjar, 40°22′27″N 5°16′03″W, 1,493 m, granite	ROS, OBL, SEM, HYB	18 (12), 18 + 1B (4), 19 (3)	15	15	–	–	–
73[a]	Ávila: Puerto de Tornavacas, 40°16′07″N 5°39′07″W, 1,284 m, granite	ROS, OBL, SEM, HYB	18 (12), 18 + 1B (4), 19 (2)	10	–	4	4	4
74[a]	Ávila: Puerto del Pico, 40°19′69″N 5°00′21″W, 1,352 m, granite	ROS, OBL, SEM, MEL, HYB	18 (45), 18 + 1B (5), 19 (5)	15	15	35	35	35
75[a]	Ávila: Puerto de Mijares, 40°19′21″N 4°48′72″W, 1,570 m, granite	ROS, OBL, HYB	18 (45), 18 + 1B (2), 19 (5)	15	15	35	35	35
76[a]	Salamanca: Navacarro, 40°23′10″N 5°42′88″W, 1,149 m, granite	ROS, OBL, SEM, HYB	18 (37), 18 + 1B (1), 19 (3)	10	–	30	30	30
77[a]	Salamanca: between Navacarro and Candelario, 40°22′11″N 5°43′83″W, 1,243 m, granite	ROS, OBL, SEM, HYB	18 (31), 18 + 1B (6), 19 (2)	15	15	18	18	18
78[a]	Salamanca: Candelario, 40°22′86″N 5°44′50″W, 1,133 m, granite	ROS, OBL, SEM, HYB	18 (9), 18 + 1B (4), 19 (1)	10	–	–	–	–
Total				734	519	365	375	382

Pop. Population, *N* number of individuals studied, *KF* karyotype formulae, *KM* karyotype morphology, *M* meiosis, *PS* pollen stainability, *F* fecundity

[a] The number of individuals in parenthesis: $2n = 2x = 18$: Pop. 70, ROS (12), OBL (10), HYB (12); Pop. 71, ROS (5), OBL (5), HYB (5); Pob 72, ROS (3), OBL (4), SEM (2) HYB (3); Pob 73, ROS (3), OBL (3), SEM (2), HYB (4); Pob 74, ROS (13), OBL (14), SEM (3), MEL (1), HYB (14); Pob 75, ROS (15), OBL (15), HYB (15); Pob 76, ROS (13), OBL (11), SEM (2), HYB (11); Pob. 77, ROS (10), OBL (9), SEM (2), HYB (10); Pob. 78, ROS (2), OBL (2), SEM (1), HYB (4); $2n = 2x = 18+1B$: Pob. 70, ROS (3), OBL (3), HYB (3); Pob 71, HYB (3); Pob. 72, (ROS (1), OBL (1), HYB (2); Pob. 73, ROS (1), OBL (1), HYB (2); Pob. 74, ROS (1), OBL (1), HYB (3); Pob. 75, HYB (2); Pob. 76, HYB (1); Pob. 77, ROS (2), HYB (4); Pob. 78, ROS (2), OBL (1), HYB (1); $2n = 2x = 19$: Pob. 72, ROS (1), OBL (1), HYB (1); Pob. 73, HYB (2); Pob. 74, ROS (2), OBL (1), HYB (2); Pob. 75, ROS (1), OBL (1), HYB (3); Pob. 76, HYB (3); Pob. 77, OBL (1), HYB (1); Pob. 78, ROS (1)

indices of chromosome asymmetry [according to Romero Zarco (1986)] and the karyotype asymmetry [following the classification of Stebbins (1971)] were established from the mitotic plates of 519 individuals, except for the karyotype formula, which was established for 719 individuals (see Table 1). In each individual, three metaphase plates with similar degrees of chromosome contraction were studied.

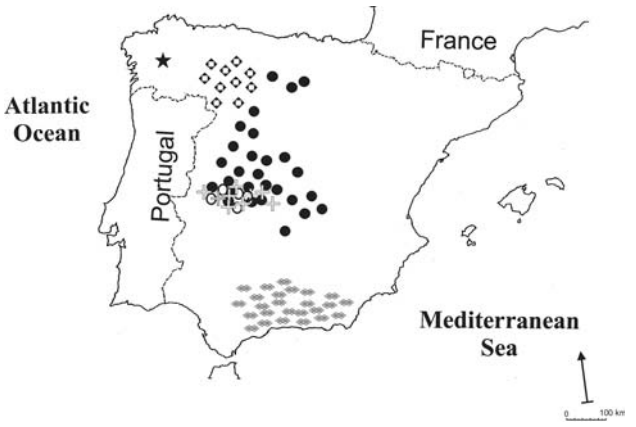


Fig. 1 Geographical distribution of the studied populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (*filled circle*), *S. oblongifolia* (*open circle*), *S. semidentata* subsp. *semidentata* (◈), *S. semidentata* subsp. *melidensis* (★), *S. canescens* (⋇), hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (╬)

The meiotic configurations (bivalent and multivalent frequencies) were determined following the classification of Jackson and Casey (1982), and frequencies of terminal and interstitial chiasmata followed the classification of Sybenga (1975). The study was carried out in 365 individuals (Table 1). In each individual, three to five meiocytes (1,110 meiocytes in total) were analysed.

Pollen fertility, indicated by pollen stainability, was estimated by counting 300–400 mature pollen grains in each of 375 plants (Table 1) using cotton-blue stain. The total quantity of sterile pollen was estimated as the sum of the numbers of aborted pollen grains and of non- or lightly-stained pollen grains. The pollen grains that showed cytoplasm uniformly stained dark blue were considered fertile.

Fecundity

The numbers of flowers and of achenes per capitulum were determined. For each character, three observations were carried out in 382 individuals (Table 1). The percentage of fruiting was calculated as (number of achenes per capitulum × 100/number of flowers per capitulum).

Statistical methods

Each specimen measured was treated as an independent operational taxonomic unit (OTU) for the whole statistical test, although dissimilarity between groups of OTUs (taxa and populations) was also measured.

A principal component analysis (PCA) was employed to explore the correlation structure of characters studied in meiosis and to reduce the number of studied characters. Nested ANOVA and nested MANOVA methods were employed to analyse the variation within and between taxa and within the hybrid complex of the PCA factors of meiotic characteristics and of pollen stainability in the former, and karyotype characteristics and fecundity in the latter. Multiple regression was applied to evaluate the effects of multivalent frequency at diakinesis and of abnormal anaphase I stages (simple and double bridges without fragments and delayed disjunction of homologous and non-homologous chromosomes) on pollen stainability.

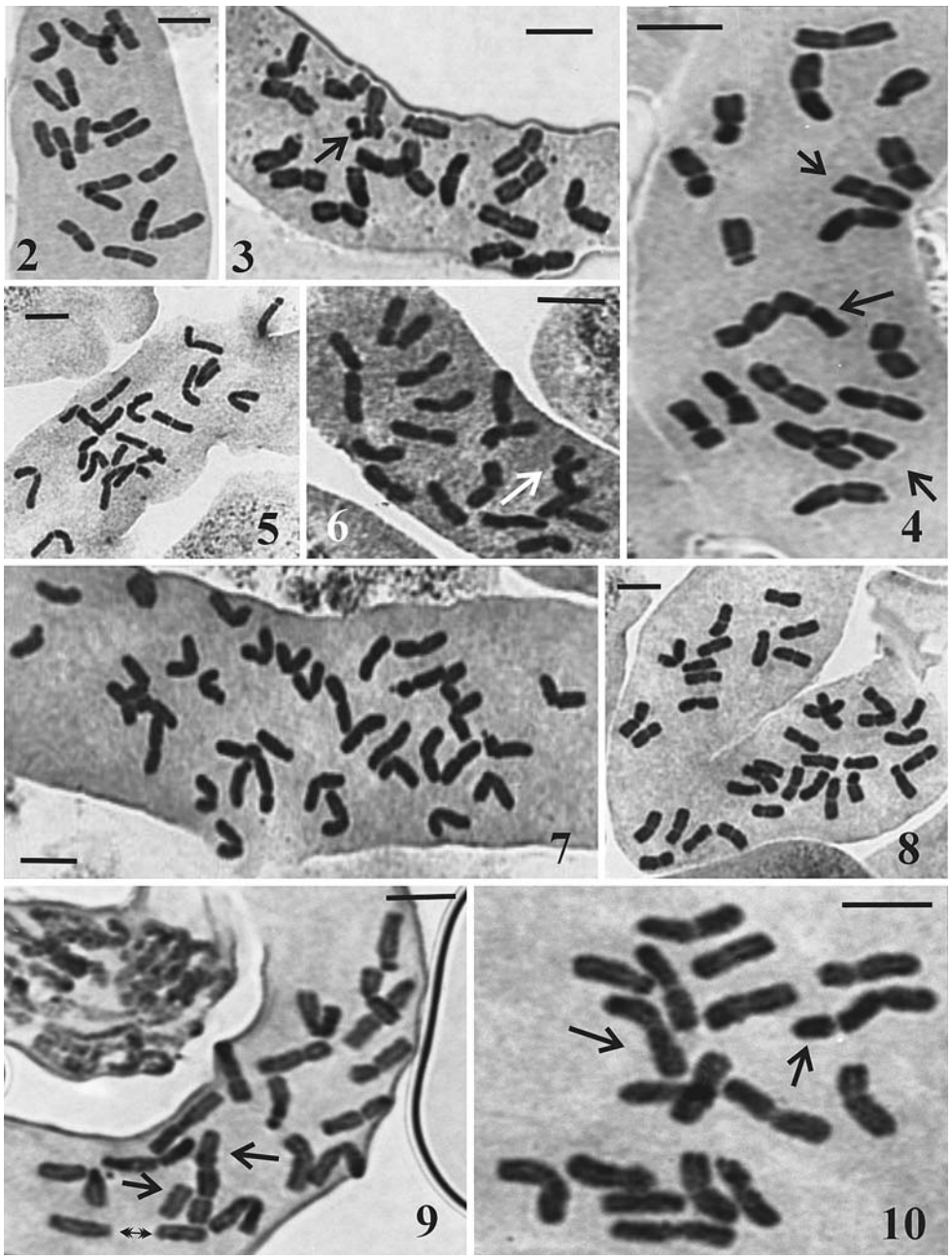
The statistical methods were applied after ensuring that data distribution requirements were met for (1) multivariate (MANOVA) or univariate (ANOVA) normality by means of the Shapiro–Wilk contrast, (2) the homogeneity of variances by means of the Barlett-Box contrast in the multivariate models and the Levene test in the univariate models (Dytham 2003; Grafen and Hails 2003) and (3) the presence of rare values or outliers, which were detected graphically, MANOVA being especially sensitive to them. The characters were square-root-transformed prior to the analysis to increase the homogeneity of variance. For the statistical analysis, the statistics package used was STATISTICA version 6.0. Results were deemed significant if the probability of the null hypothesis was less than 0.05.

Results

Geographical distribution and ecology

Santolina oblongifolia, *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* are endemic to the Iberian Peninsula, while *S. canescens* is endemic to the Iberian Peninsula and the north of Morocco. *Santolina oblongifolia* is located in the Iberian Central Systems (Gredos-Béjar-Gata-Candelario-Alberca-Tormantos massif) on granite substrate, at 1,000–2,360 m, the mean being 1,662.50 ± 543.71 m. This species coexists and hybridises with *S. rosmarinifolia* subsp. *rosmarinifolia* in the Gredos-Candelario-Béjar-Tormantos massif, at 900–1,800 m, the mean being 1,253.94 ± 210.23 m.

Santolina canescens inhabits the southern portion of the Iberian Peninsula, from Almería (Sierra de Los Filabres) to Cádiz (Sierra de Grazalema) provinces. It is found at 400–2,400 m, the mean being 1,267.62 ± 440.70 m, usually on limestone, marl–limestone, marl, schist and quartzite and conglomerates (Table 1). *Santolina semidentata* subsp. *semidentata* is located in the north-west of the Iberian Peninsula [León, south of Lugo, north-eastern portion of Ourense, north of Zamora and Tras-os-Montes provinces, and in the Brezo massif (Palencia)]. It is found at an altitude of 400–1,400 m, the mean being 938.00 ± 245.19 m, usually on slates and quartzite or schist. This taxon is



Figs. 2-10 Somatic metaphases in five taxa of the *S. rosmarinifolia* aggregate, the hybrid complex *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and in their putative hybrids. **Fig. 2** $2n = 2x = 18 = 14m + 2sm + 2st$ (Almería: Calar Alto). **Fig. 3** $2n = 2x = 18 + 1B = 14m + 2sm + 2st + 1B$ (Salamanca: between Navacarro and Candelario). The *arrow* indicates a B chromosome. **Fig. 4** $2n = 2x = 19 = 15m + 2sm + 2st$ (Salamanca: Navacarro). The *arrows* indicate trisomy in pair IV. **Fig. 5** $2n = 2x = 18 = 14m + (1\ m - 1sm) + (1m - 1st)$ (La Coruña: Santiso). **Fig. 6** $2n = 2x = 18 = 12m + (1m - 1sm) + (1m - 1st) + 2st + 1B$ (La Coruña: Santiso). The *arrow* indicates a B chromosome. **Fig. 7** $2n = 4x = 36 = 24m + 8sm + 4st$ (Toledo: Mocejón). **Fig. 8** $2n = 4x = 36 = 28m + 4sm + 4st$ (Almería: Esfiliana). **Fig. 9** $2n = 2x = 18$. The *double arrow* indicates a metacentric chromosome break for the centromere due to squash process. The *thick arrows* indicate that one homologue has lost the short arm, and this arm is fused with the long arm of the other homologue in pair V. The remaining long arm is telocentric (Ávila: Puerto de Tornavacas). **Fig. 10** $2n = 2x = 18$. The *arrows* indicate one pair of homologous submetacentric chromosomes showing additional fragments, one of them in the short arm of one chromosome and one in the long arm of the homologue (Salamanca: Candelario)

sympatric for the eastern portion with *S. rosmarinifolia* subsp. *rosmarinifolia* and for the western portion with *S. semidentata* subsp. *melidensis*. The latter is located in Santiso, in the area of Barazón (La Coruña province) on serpentine soils, at 410 m. This area forms part of the central Galician massif (and coincides with the border

Table 2 Karyotype variability of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens* and in the hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids by means of nested MANOVA aimed at detecting variation within and between taxa, within the hybrid complex, and within and between taxa with regard to chromosome pairs

Source of variation	Multivariate analysis				Univariate analysis						
	Wilk's λ	F	dfe	dfr	dfe, dfr	LBC F(VCP)	LBL F(VCP)	LTC F(VCP)	A_1 F(VCP)	A_2 F(VCP)	r F(VCP)
Variation within and between taxa											
BTX	0.62	10.52****	20	1,364.08	4, 415	23.06****	28.23****	44.62****	5.50***	3.52*	–
						(13.00)	(14.30)	(19.00)	(2.40)	(3.10)	–
BPT	0.42	3.20****	120	2,024.83	24, 415	5.80****	7.32****	10.76****	2.92****	0.87 ns	–
						(21.10)	(25.40)	(31.90)	(11.10)	–	–
Variation within the hybrid complex											
BP	0.35	4.46****	16	174.77	4, 60	2.54*	8.00****	10.50****	8.42****	0.07 ns	–
						(1.50)	(34.80)	(28.40)	(36.20)	–	–
BTP	0.40	1.48*	40	217.99	10, 60	3.82***	0.93 ns	3.04**	0.91 ns	1.04 ns	–
						(37.80)	(2.45)	(21.90)	(0.94)	(0.90)	–
Variation within and between taxa with regard to chromosome pairs											
BTX	0.58	34.25*	20	3,888.03	5, 1,175	73.05	102.91*	147.12*	–	–	0.52 ns
						(8.90)	(24.90)	(24.00)	–	–	–
BPT	0.69	5.64*	80	4,625.81	20, 1,175	11.85*	14.16*	20.53*	–	–	1.08 ns
						(9.20)	(12.50)	(10.20)	–	–	–
RTP	0.10	4.30*	836	4,690.34	209, 1,175	14.24*	1.84*	5.66*	–	–	11.93*
						(62.70)	(7.70)	(28.80)	–	–	(64.60)

dfe Degree of freedom of the effect, *dfr* degree of freedom of the error, *LBL* mean length of the long arm, *LBC* mean length of the short arm, *LTC* mean of the total length of the chromosome, A_1 intrachromosomal asymmetry index, A_2 interchromosomal asymmetry index, *BTX* between taxa, *BPT* between populations within each taxon, *BP* between populations, *BTP* between taxa within each population, *RTP* between chromosome pairs in each taxon and population, *VCP* variance components (%)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, *ns* $P > 0.05$

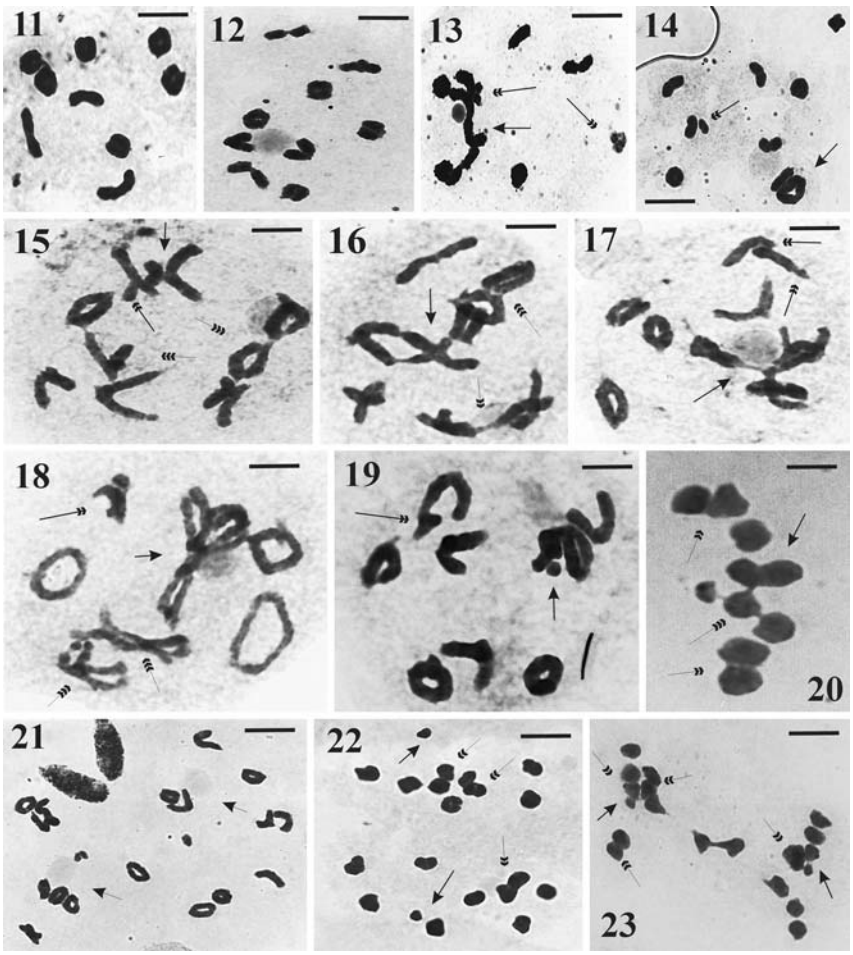
between La Coruña, Lugo and Pontevedra provinces) (Rodriguez-Oubiña and Ortiz 1993). Figure 1 shows the approximate positions of the studied plants.

Somatic chromosome number and karyotype morphology

Santolina oblongifolia, *S. semidentata* subsp. *semidentata*, *S. canescens* and *S. semidentata* subsp. *melidensis* are diploids with $2n = 2x = 18$ (Table 1, Fig. 2). *Santolina oblongifolia*, *S. rosmarinifolia* subsp. *rosmarinifolia* and their putative hybrids in the hybrid complex are also diploids with $2n = 2x = 18$, 18 + 1B (Fig. 3), 18 + 2B, 18 + 4B (Table 1), 19 with trisomy in pair VI (Fig. 4) and 20 (Table 1). The chromosome numbers are listed in Table 1. B-chromosomes are smaller than the smallest A-chromosomes, usually metacentric (Fig. 3), rarely subtelocentric and variable at the individual level (Table 1). The karyotype formula is usually 14m + 2sm + 2st (Fig. 2), although it is variable in *S. semidentata* subsp. *melidensis*, being 14m + 2sm + 2st in ten individuals, 14m + (1m − 1sm) + (1m − 1st) in nine individuals (Fig. 5) and 12m + (1m − 1sm) + (1m − 1st) + 2st + 1B in five individuals (Fig. 6). In the diploid population of *S. rosmarinifolia* subsp. *rosmarinifolia* in Mocejón (Toledo), two tetraploid individuals with a karyotype formula $2n = 4x = 36 =$ 24m + 8sm + 4st were found (Fig. 7). Two and four tetraploid individuals of *S. canescens* were found in the diploid populations of Esfiliana (Almería) and Fuensanta de Martos (Jaén), respectively, with a karyotype formula $2n = 4x = 36 =$ 28m + 4sm + 4st (Fig. 8).

In one individual of a putative hybrid from Puerto de Tornavacas, the short arm of one of pair V was transposed to the long arm of the other homologue; the remaining long arm is telocentric (Fig. 9). Another individual from Candelario showed one pair of homologous metacentric chromosomes to have a secondary constriction in the long arm (Fig. 10).

Satellite number vary in the range 0–4 and are usually on two pairs of metacentric or one pair of metacentric and one of submetacentric chromosomes, except in *S. semidentata* subsp. *melidensis*, in which they vary in the range 0–2,



Figs. 11-23 Chromosome pairing configurations at diakinesis, metaphase and anaphase I in five taxa of the *Santolina rosmarinifolia* L. aggregate. **Fig. 11** Metaphase I, $2n = 2x = 18$, 6 OII + 3 CII (Ávila: La Mira). **Fig. 12** Metaphase I, $2n = 2x = 18$, 2 OII + 7 CII, three bivalents associated with the nucleolus are observed (Almería: Esfiliana). **Fig. 13** Diakinesis, $2n = 2x = 18 + 4B$, 3 OII + 4 CII + 1 CIV + 2B II. The *thin arrow* indicates quadrivalent chain associated with the nucleolus, and three bivalents are associated with it; the *double arrows* indicate B bivalents (La Coruña: Barazón). **Fig. 14** Diakinesis, $2n = 2x = 20 + 2B$, 2 OII + 6 CII + 1 OIV + 1B II. The *thin arrow* indicates quadrivalent ring; the *double arrow* indicates B bivalent; two bivalents associated with the nucleolus are observed (Ávila: between Navacarro and Candelario). **Fig. 15** Diakinesis, $2n = 2x = 19$, 3 OII + 5 CII + 1 CIII. The *thin arrow* indicates trivalent chain; the *double arrow* indicates Y-shape open bivalent; the *triple arrows* indicate adhesions of bivalents (one and five bivalents are formed by subtelocentric and metacentric chromosomes, respectively); one of them is associated with the nucleolus (Ávila: near La Plataforma de Gredos). **Fig. 16** Diakinesis, $2n = 2x = 19$, 1 I + 4 CII + 1 OIV + 1 CVI. The *thin arrow* indicates heteromorphic hexavalent chain (metacentric-subtelocentric) with pericentric inversion that includes the centromere of one chromosome, and one univalent (subtelocentric) in parallel position with its homologue; the *double arrow* indicates adhesions of bivalent, and they are associated with the nucleolus; the *triple arrow* indicates quadrivalent ring (Ávila: La Aliseda de Tormes). **Fig. 17** Diakinesis, $2n = 2x = 18$, 2 I + 3 OII + 3 CII + 1 CIV. The *double arrows* indicate secondary constriction. The *thin arrow* indicates quadrivalent chain and two overlap bivalents associated with the nucleolus; one of them (to the *left*) shows adhesion with one chromosome of the quadrivalent chain (Ávila: 17 km from El Barco de Ávila). **Fig. 18** Diakinesis, $2n = 2x = 18$, 2 OII + 2 CII + 1 CIX − 1frag + 1frag with satellite. The *thin arrow* indicates the nonevalent chain less a fragment associated with the nucleolus; the *double arrow* indicates chromosome fragment with satellite; the *triple arrows* indicate paracentric inversions (Ávila: 17 km from El Barco de Ávila). **Fig. 19** Diakinesis, $2n = 2x = 18$, 4 OII + 3 CII + 1 CII − 1frag + 1 CII + 1frag. The *thin arrow* indicates a chromosome fragment due to double breakage in the chromosome arm. The *double arrow* indicates that the other chromosome fragment is fused with the open bivalent (Ávila: El Barco de Ávila). **Fig. 20** Metaphase I, $2n = 2x = 18 + 2B$, 6 OII + 1 CII + 1 OIV + 1B II. The *thin arrow* indicates quadrivalent ring; the *double arrows* indicate adhesion of bivalents; the *triple arrow* indicates a ring of bivalents showing adhesions with a bivalent ring, B bivalent and with a quadrivalent ring (Ávila: 17 km from El Barco de Ávila). **Fig. 21** Diakinesis, $2n = 4x = 36 + 4B$, 9 OII + 9 CII + 2B II. The *arrows* indicate two nucleoli, one of them with two bivalents associated and the other with three bivalents (two of them with adhesion) and one B bivalent chromosome associated (Toledo: Mocejón). **Fig. 22** Anaphase I. The *arrows* indicate the normal segregation of B chromosomes; the *double arrows* indicate delayed disjunction of homologous chromosomes (Ávila: Navarredonda de Gredos). **Fig. 23** Anaphase I with double chromatin bridges without fragment. The *thin arrows* indicate the normal segregation of B chromosomes and their adhesion with an A chromosome. The *double arrows* indicate a delayed disjunction of homologous and non-homologous chromosomes (Ávila: near La Plataforma de Gredos)

usually on two metacentrics or one metacentric and one subtelocentric, or one metacentric and one submetacentric chromosomes. In the hybrid complex, the satellite number varies from 0 to 5, usually on one submetacentric and one or two (two only in *S. oblongifolia*) pairs of metacentric. The satellite is usually observed in the short arm, except in one individual of *S. oblongifolia*, which shows the long arm of the chromosome with one satellite.

The mean length of the short arm, the long arm and total length of the chromosome are higher than in the other taxa in *S. semidentata* subsp. *melidensis* and lower in *S. oblongifolia* (see Table 6 in the Appendix). They are also higher in *S. rosmarinifolia* subsp. *rosmarinifolia* and in *S. oblongifolia* of the hybrid complex than in the other populations of these taxa. The A_1 and A_2 show a narrow range of variation within and between taxa (see Table 6 in the Appendix), but the mean values of A_1 are higher in *S. semidentata* subsp. *melidensis* than in the other taxa.

Nested MANOVA shows significant differences for all the sources of variation analysed (Table 2). Variance components indicate that no particular chromosome characteristics contribute strongly to taxon differentiation. The same analysis within the hybrid complex (Table 2) shows statistical homogeneity for karyotype morphology between taxa, except for length of the short arm and total length of the chromosome.

Nested MANOVA, in relation to chromosome pairs (Table 2), showed statistical heterogeneity between and within taxa for karyotype morphology, except for chromosome ratio between taxa and between populations within each taxon. Variance components indicate that the variation in length of the short arm and chromosome ratio occurs principally between chromosome pairs in each individual. The post-hoc test indicates that the chromosome ratio, which defines each type of chromosome, shows significant differences (for the error term $df = 1{,}175$; $\mathrm{MS} = 0.46$, $P < 0.0001$) between metacentric and subtelocentric chromosome only. This indicates that the submetacentric chromosomes are not statistically distinguishable from the others.

Meiotic configuration and chiasma frequency

The study shows the predominance of bivalent configurations over multivalent configurations (see Table 7 in the Appendix, Figs. 11–21). Meiotic configurations above the bivalent level are observed in all of the taxa except in *S. oblongifolia* and populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif and Álava, Ávila and Burgos province (see Table 7 in the Appendix). Meiotic configurations are very variable in *S. rosmarinifolia* subsp. *rosmarinifolia*. The populations from the Guadarrama massif and Álava, Ávila and Burgos province show normal meiosis, whereas those from Toledo, Salamanca, Zamora and the Gredos massif show abnormal meiosis (see Table 7 in the Appendix).

Ring bivalents predominate over open bivalents in *S. oblongifolia* and the populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif and Álava, Ávila and Burgos province as well as the tetraploid cytotypes of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* (see Table 7 in the Appendix, Fig. 11). The opposite occurs in the populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora and the Gredos massif and in diploid cytotypes of *S. canescens* (Fig. 12). However, in the putative hybrids and in the tetraploid cytotypes of *S. canescens*, the frequencies of ring and open bivalents is similar. Chain quadrivalents (Figs. 13, 14, 16, 17, 20) and hexavalents (Fig. 16) are the most frequent configurations. Trivalent rings, open trivalents and chains of pentavalents are observed in the putative hybrids only (see Table 7 in the Appendix). Octovalent and nonevalent (see Table 7 in the Appendix, Fig. 18) chains are observed in the hybrid complex for *S. rosmarinifolia* subsp. *rosmarinifolia* and for the putative hybrids. Chromosomes with translocated segments are observed in the hybrid complex only (Fig. 19).

B chromosomes are observed in all the taxa, except in *S. oblongifolia*, in the populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Guadarrama massif and Álava, Ávila and Burgos province and in diploid cytotypes of *S. canescens* (Table 1, see Table 7 in the Appendix). They show bivalent association in diakinesis (Fig. 13, 14, 20, 21) and, in one individual only, associate with bivalent ring in metaphase I (Fig. 20). There are no differences in staining

Table 3 Variability in pollen stainability by means of nested ANOVA aimed at detecting variation within and between taxa and within the hybrid complex

Source of variation	dfe, dfr	*F*	VCP
Variation within and between taxa			
BTX	4, 450	5.15**	1.70
BPT	14, 450	2.86**	3.60
BITP	197, 450	1.42*	11.30
Variation within hybrid complex			
PB	5, 299	5.85***	2.30
BTP	7, 299	2.93*	6.10
BIPT	128, 299	1.14 ns	3.90

BTX Between taxa, *BPT* between populations within each taxon, *BITP* between individuals in each taxon and population, *PB* between populations, *BTP* between taxa within each population, *BIPT* between individuals in each population and taxon, *dfe* degree of freedom of the effect, *dfr* degree of freedom of the error, *VCP* variance components (%)

*$P < 0.05$,**$P < 0.001$, ** $P < 0.0001$, *ns* $P > 0.05$

potential and the segregation at anaphase I is normal (Figs. 22, 23), but usually they show adhesion with A chromosomes in anaphase I.

Chiasmata are mostly terminal (see Table 8 in the Appendix). Terminal chiasmata are fewer in *S. canescens* than in the other taxa, but the frequency of interstitial chiasmata is higher in this taxon.

All of the taxa show abnormal anaphase I (see Table 9 in the Appendix, Figs. 22, 23), except for *S. oblongifolia* and populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Guadarrama massif and Álava, Ávila and Burgos province. The frequency of simple chromosome bridges is higher than that of double chromosome bridges, both without fragments. Double bridges are not observed in *S. semidentata* subsp. *melidensis* and *S. canescens*. The values for frequency of simple bridges and of the homologous and non-homologous chromosomes that show delayed disjunction in anaphase I are similar in *S. rosmarinifolia* subsp. *rosmarinifolia* (populations of Toledo, Salamanca and Zamora), in *S. canescens* and in *S. oblongifolia* from the hybrid complex (see Table 9 in the Appendix).

Bivalent adhesion and delayed disjunction of the homologous and non-homologous chromosomes are observed in all of the taxa in diakinesis and anaphase I respectively (Figs. 13, 15–17, 19–23), except for *S. oblongifolia* and populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Guadarrama massif and Álava, Ávila and Burgos province.

PCA shows that the first four factors account for 61.66% of the variance. Ring bivalents ($r = 0.65$), rod bivalents ($r = -0.81$), total chiasma frequency ($r = 0.83$) and frequency of terminal chiasmata ($r = 0.78$) show moderate to strong correlation with factor 1 (eigenvalue = 3.58, 23.80% of the variance). Chain quadrivalents ($r = 0.53$), chain hexavalents ($r = 0.61$), chain nonevalents ($r = 0.54$), simple ($r = 0.69$) and double ($r = 0.72$) chromosome bridges without fragments and the homologous and non-homologous chromosomes that show delayed disjunction in anaphase I ($r = 0.69$) show moderate correlation with factor 2 (eigenvalue = 2.70, 18.00% of the variance). Chain trivalents ($r = 0.62$) and univalents ($r = 0.86$) show moderate to strong correlation with factor 3 (eigenvalue = 1.62, 10.85% of the variance), while the frequency of interstitial chiasmata ($r = 0.87$) shows strong correlation with factor 4 (eigenvalue = 1.33, 8.90% of the variance). Nested ANOVA (see Table 10 in the Appendix) shows that the variation in PCA factors is not great for any source of the variation analysed. The meiosis of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and of the putative hybrids is not statistically distinguishable between these taxa within the hybrid complex, except for factor 1 (see Table 10 in the Appendix). Interstitial chiasmata (factor 4) is mostly variable between individuals in the populations, whereas the remaining characters are mostly variable between populations.

Pollen stainability

Pollen is fertile, with a broad range of variation (see Table 11 in the Appendix). Pollen stainability is lower in the putative hybrids than in the other taxa. Nested ANOVA (Table 3) shows that the variation between populations and between individuals in the populations is higher than the variation between taxa. The variation between taxa within populations in the hybrid complex is higher than the

Table 4 Summary of the fecundity of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	*N*	NFPC			NAPC			PFPC		
		Range	Mean ± SD	CV (%)	Range	Mean ± SD	CV (%)	Range	Mean ± SD	CV (%)
ROS-CN	151	190–320	240.02 ± 50.21	20.92	20–158	143.20 ± 53.25	37.18	10.52–46.87	59.66 ± 22.14	37.11
ROS-T	201	200–241	212.51 ± 51.12	24.08	63–132	110.15 ± 20.12	18.26	31.5–54.77	51.83 ± 27.10	53.69
OBL	93	70–177	90.21 ± 25.74	28.53	20–83	59.86 ± 20.14	33.64	28.57–46.89	66.35 ± 22.36	33.70
SEM	93	172–315	233.78 ± 60.87	26.03	89–189	170.58 ± 40.51	23.74	51.74–60.00	72.96 ± 15.28	20.97
MEL	46	64–158	95.19 ± 29.78	31.28	30–79	67.49 ± 23.26	34.46	46.87–50.00	70.90 ± 15.33	21.62
CAN	82	183–373	253.48 ± 50.15	19.78	98–190	175.28 ± 44.10	25.16	53.55–50.93	69.15 ± 29.59	42.79
MIX										
ROS-G	257	150–358	232.58 ± 70.23	30.19	35–198	156.47 ± 50.27	32.12	23.33–55.31	67.25 ± 27.89	41.47
OBL-G	76	100–378	208.42 ± 86.13	41.32	60–278	119.47 ± 72.49	60.67	55.25–73.54	54.36 ± 13.42	24.68
HYB	107	225–240	237.25 ± 33.83	14.25	77–177	130.20 ± 29.85	22.92	34.22–73.75	54.87 ± 10.78	19.64

N Number of samples studied, *CN* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif, Álava, Ávila and Burgos provinces, *T* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora provinces, *NFPC* number of flowers per capitulum, *NAPC* number of achenes per capitulum, *PFPC* percentage of fructification per capitulum, *CV* coefficient of variation, *SD* standard deviation

Table 5 Variability of fecundity by means of nested MANOVA aimed at detecting variation with and between taxa and within hybrid complex

Source of variation	Multivariate analysis ($P < 0.0001$)				Univariate analysis ($P < 0.0001$)			
	Wilk's λ	F	dfe	dfr	dfe, dfr	NFPC F(VCP)p	NAPC F(VCP)p	PFPC F(VCP)p
Variation within and between taxa								
BTX	0.05	207.30	12	1185.58	4, 450	497.32 (36.70)	214.39 (21.40)	46.13 (22.60)
BPT	0.02	91.18	42	1329.75	14, 450	84.30 (20.90)	51.73 (17.50)	95.02 (31.10)
BITP	0.003	12.84	591	1344.91	197, 450	11.34 (32.70)	10.87 (46.60)	14.17 (55.80)
Variation within hybrid complex								
PB	0.07	84.82	15	820.28	5, 299	84.06 (13.70)	41.71 (11.90)	19.06 (0.70)
BTP	0.09	49.99	21	853.37	7, 299	21.75 (5.09)	4.50 (10.15)	15.59 (2.00)
BIPT	0.003	14.23	384	891.85	128, 299	13.17 (64.70)	11.80 (68.40)	12.47 (76.70)

BTX Between taxa, *BPT* between populations within each taxon, *BITP* between individuals in each taxon and population, *PB* between populations, *BTP* between taxa within each population, *BIPT* between individuals in each population and taxon, *NFPC* number of flowers per capitulum, *NAPC* number of achenes per capitulum, *PFPC* percentage of fructification per capitulum, *dfe* degree of freedom of the effect, *dfr* degree of freedom of the error, *VCP* variance components (%)

variation between populations and between individuals within populations (Table 3). In general, the variation is not great for any source of variation analysed.

Multiple regression analysis shows that the effect of multivalent frequency and frequency of abnormal anaphase I (simple and double bridges and delayed disjunction of homologous and non-homologous chromosomes) on pollen stainability is moderate and statistically significant (adjusted $R^2 = 0.66$, $F_{4,1105} = 557.56$, $P < 0.0001$). This indicates that the pollen stainability of individuals with meiotic irregularities is lower than that of individuals with normal meiosis, as might be expected.

Fecundity

Flower and achene numbers per capitulum are lower in *S. oblongifolia* and in *S. semidentata* subsp. *melidensis* than in the other taxa (Table 4). The individuals of *S. oblongifolia* from the hybrid complex show higher flower and achene numbers per capitulum than the remaining populations of *S. oblongifolia* (Table 4). All the taxa show similar percentages of fruiting. Nested MANOVA (Table 5) shows that the flower and achene numbers per capitulum and percentage of fruiting are significantly different for all the sources of variation analysed, but the variation between individuals in the populations is great. The same occurs within the hybrid complex (Table 5).

Discussion

Cytogenetics

The base chromosome number in all of the studied taxa is $x = 9$, which agrees with that proposed for the genus *Santolina* (Valdés-Bermejo et al. 1981), and for *S. pectinata*, *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. ageratifolia* (Rivero-Guerra 2008a, b, c respectively) and *S. impressa* (Rivero-Guerra 2009a). The mean chromosome length and the mean lengths of the long and short arms of *S. semidentata* subsp. *melidensis* are higher than those found in diploid and polyploid taxa (Rivero-Guerra 2008a, b, c; Rivero-Guerra 2009a), but A_1 is higher in polyploids such as *S. ageratifolia* (0.35; Rivero-Guerra 2008c), the tetraploid cytotypes of *S. pectinata* (0.36; Rivero-Guerra 2008a) and *S. rosmarinifolia* subsp. *arrabidensis* (0.37; Rivero-Guerra 2008b) than in diploids. The karyotypes analysed correspond to those found by Valdés-Bermejo et al. (1981) for *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata S. canescens* and *S. oblongifolia*. In general, the karyotypes show low values of asymmetry, as is common in the tribe *Anthemideae* (Schweizer and Ehrendorfer 1983).

The results support the hypothesis that claims specific and infraspecific karyotype stability for these taxa within and outside the hybrid complex, as their karyotype displays a constancy of basic chromosome number, a uniformity of chromosome morphology (a high frequency of m-type chromosomes) and consequently a constancy in the karyotype formula in diploid and tetraploid taxa. This indicates that numerical chromosome changes have not been important in the evolution of the group, except in *S. canescens*, *S. pectinata* and *S. rosmarinifolia* subsp. *rosmarinifolia*, in which diploid and tetraploid cytotypes were found. The high degree of sympatry of the diploid taxa of the *S. rosmarinifolia* aggregate and the conserved general structure of their karyotype do not support the existence of major genetic barriers between these taxa (Rivero-Guerra 2009b), so hybridisation with

introgression may have played an important role in the evolution of this aggregate.

Tetraploid individuals of *S. rosmarinifolia* subsp. *rosmarinifolia* have the same karyotype formula as that of *S. rosmarinifolia* subsp. *arrabidensis* (Rivero-Guerra 2008b) and show close morphological similarity (Rivero-Guerra 2009b). Similarly, tetraploid individuals of *S. canescens* have the same karyotype formula as that of tetraploid populations of *S. pectinata* (Rivero-Guerra 2008a). The results support the recent and spontaneous origin of the tetraploid individuals within diploid populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. canescens*. Although no unreduced gametes were found in the diploid populations studied, the sexual mechanism of tetraploid formation through bilateral fusion of unreduced gametes (Harlan and deWet 1975; Bretagnolle 2001) is plausible. However, non-reduction is very rare, so the chance that a non-reduced pollen grain will fertilise a non-reduced egg cell falls almost to zero, particularly in view of the large numbers of fertile haploid gametes that are produced by diploids (P.E. Brandham, personal communication). More probably it is somatic doubling or a two-step non-reduction process involving a triploid bridge (Husband 2004).

The survival and perpetuation of these autopolyploids in their initial stages depend upon especially favourable combinations of circumstances, mainly with regard to their competitive ability, life history, demographic stochasticity, and their fitness and ability to overcome the minority disadvantage, either by replacing diploids or by spreading beyond their site of origin and establishing a new population system (Levin 1983; Felber 1991; Rodríguez 1996; Burton and Husband 2000; Stuessy et al. 2004).

The high frequency of open bivalents is indicative of a partial failure of synapsis in the chromosome arms or is due to non-formation of cross-overs in some of the arms (Jos et al. 1968; Ferreira 1985). The frequency of interstitial chiasmata is higher than that of terminal chiasmata in diploid and tetraploid cytotypes of *S. pectinata* (Rivero-Guerra 2008a) and of hexaploid *S. ageratifolia* (Rivero-Guerra 2008c), contrary occurs in the remaining taxa. The high frequency of terminal chiasmata may be the result of either a terminalisation process in the later stages of prophase or of chiasmata localisation on the distal part of the bivalent.

Adhesion of bivalents in the taxa can be attributed to (1) residual attraction between bivalents composed of genetically or structurally similar pairs of chromosomes, analogous to the attraction which gives rise to primary prophase synapsis (Darlington and Moffett 1930; Lacadena and Puertas 1969; Gupta and Roy 1973), (2) the fusion of heterochromatic portions (Thomas and Revell 1946), (3) the homology between chromosomes (Jacob 1957) or (4) a relic of prophase attraction (Riley 1960; Kempanna and Riley1964).

The variation in the number of each type of chromosome in *S. semidentata* subsp. *melidensis* and the presence of the following in the taxa can be attributed to chromosome translocation and/or chromosome inversions: (1) B chromosomes, (2) trisomic individuals, (3) fragments and (4) arm deletions, (5) chromosome fusion and (6) meiotic configuration above the bivalent level in diploid taxa. The presence of bridges and of the delayed disjunction of the homologous and non-homologous chromosomes at anaphase I is indicative of (1) heterozygous paracentric and pericentric chromosome inversions (Sybenga 1975; Stein et al. 2004), (2) pairing between chromosomes that are not completely homologous, so that there is a delay in disjunction (Raina and Khoshoo 1971), (3) delayed separation of two bivalents, perhaps due to the arrest of terminalisation of chiasmata (Jos et al.1968) or (4) breakage and reunion processes in prophase (Gustafsson 1972). The chromosome inversion and reciprocal translocations did not modify the karyotype morphology, therefore they may not have been large structural mutations.

B chromosomes are paired in diakinesis and show normal segregation at anaphase I in diploid taxa. They may arise as a consequence of the translocations or inversions in chromosomes that participate in multivalent configurations, or from the fusion of some fragments, and/or from centromeric fragments (Jackson 1965; Jones and Houben 2003; Palestis et al. 2004), or by deletion of chromosome arms (Jones 1991).

Pollen stainability and fecundity

Hybrid fitness is typically lower than that of both parental species, but often some genotypes are more fit than either parental species (e.g., Wang et al. 2006; Cozzolino et al. 2006), and fitness is frequently contingent on the environment (e.g., Grant and Grant 2002). The components of the fitness of the hybrid complex of *Santolina* are variable. Pollen stainability of the putative hybrids is lower than that of the individuals of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia* (of the hybrid complex) and of the individuals of "pure populations" of *S. oblongifolia* and *S. rosmarinifolia* subsp. *rosmarinifolia*, whereas the fecundity of the putative hybrids of this complex is higher than that of the "pure populations" of *S. oblongifolia* and similar to that of "pure populations" of *S. rosmarinifolia* subsp. *rosmarinifolia*.

Despite the deleterious effect of the abnormal meiotic chromosome behaviour on pollen stainability, the mean pollen stainability is moderate to high in these taxa. The mean pollen stainability of the diploid taxa with abnormal diakinesis and anaphase I ranged from 10.52 to 100%, whereas the mean pollen stainability of polyploid taxa

(*S. ageratifolia*, *S. rosmarinifolia* subsp. *arrabidensis* and tetraploid cytotypes of *S. pectinata*, *S. rosmarinifolia* subsp. *rosmarinifolia*, and of *S. canescens*) ranged from 40.56 to 88.00%. In these cases, there are two possibilities: that the effect of polyploidy on pollen stainability is higher than that of meiotic irregularities in diploid taxa, or that the effect of translocation and inversions on pollen stainability is higher in polyploids than in diploids. The second hypothesis is supported by the results in tetraploid cytotypes of *S. pectinata* (51.95%) (Rivero-Guerra 2008a) and in hexaploid *S. ageratifolia* (40.56%) (Rivero-Guerra 2008c). However, the frequency of interstitial chiasmata in the diploid taxa studied here is low, and they show moderate fructification percentages, suggesting that the cross-over frequency within the inverted segment is moderate to low and consequently a moderate-to-low number of duplication-deficiency gametes has been formed. The results suggest that inverted segments are located mostly in the interstitial region.

Morphological arguments and diversification of the taxa

Rieseberg and Ellstrand (1993) and Rieseberg (1995) have demonstrated that hybridisation does not always result in morphological intermediacy, when (1) introgression is extensive, (2) the hybridisation process happened long ago and (3) the expression of several morphological traits is not under additive genetic control. The presence of multivalent configurations in diakinesis and an abnormal anaphase I suggest a introgressive hybridisation process in *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens*, and in some populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora provinces. The back-crossing of the putative hybrids with *S. rosmarinifolia* subsp. *rosmarinifolia* yields individuals morphologically similar to *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis* and *S. rosmarinifolia* subsp. *rosmarinifolia* (Rivero-Guerra 2009b). The frequency of phenotypes such as *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* is low in the hybrid complex, in particular that of the phenotypes such as *S. semidentata* subsp. *melidensis* (one plant). The low frequency of lower and middle spatulate leaves in *S. semidentata* subsp. *semidentata*, the low frequency of narrowly spatulate lower leaves and the presence of three rows of involucral bracts in *S. semidentata* subsp. *melidensis* suggest that both taxa are closely related to *S. oblongifolia* (Rivero-Guerra 2009b). In addition, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis* and the populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora provinces have the same qualitative characteristics of the appendage of the involucral bracts of *S. oblongifolia* (Rivero-Guerra 2009b).

The phenotypes with close morphological similarity to *S. rosmarinifolia* subsp. *rosmarinifolia* extend over the northwest (Salamanca, Valladolid and Zamora) and centre (Toledo and Ciudad Real) of the Iberian Peninsula. In central Spain, these phenotypes co-exists with *S. semidentata* subsp. *semidentata* and *S. rosmarinifolia* subsp. *rosmarinifolia*, and for the south-eastern portion of its distribution (Ciudad Real) it is also sympatric with *S. pectinata.* The morphology and development of the seedlings and the quantitative and qualitative characters of the populations of *S. canescens* suggest that they may have arisen by hybridisation between plants of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora and plants of *S. pectinata* (Rivero-Guerra, unpublished data).

Hybridisation is not likely to be a common adaptive mechanism and this phenomenon per se is not necessarily adaptive (Ellstrand et al. 1996). According to Barton (2001), for hybridisation to contribute to adaptation, the fit hybrid genotypes must escape from "the mass of unfit recombinants" present in a hybrid population and colonise novel habitats (Rieseberg 2001; Baack and Rieseberg 2007). The introgressive hybrid, with close morphological similarity to *S. rosmarinifolia* subsp. *rosmarinifolia*, probably "escaped" from the hybrid swarms and become established as monotype populations or cohabits in the same territory with *S. rosmarinifolia* subsp. *rosmarinifolia*, usually on basic substrates in central Spain. However, *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* live respectively on slate/quartzite and serpentine in the north-west of the Iberian peninsula. The results suggest that the introgression of advantageous alleles (Kim and Rieseberg 1999) is probably the "escape" mechanism for these taxa.

Conclusions

Chromosome number, karyotype formula, chromosome size and meiotic behaviour indicate a very close relationship between these taxa (1). The results support the recent and spontaneous origin of autopolyploid individuals within diploid populations of *S. canescens* and *S. rosmarinifolia* subsp. *rosmarinifolia* (2) and support a tendency of the taxa towards conserving the general structure of the karyotype (3). Chromosomal rearrangements have a negative effect on pollen stainability (4). The mean value of fructification percentage is moderate in these taxa (5). The presence of multivalent configurations in diakinesis and bridges and delayed disjunction of the homologous and non-homologous chromosomes at anaphase I in *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis* and in populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Béjar-Gredos-Tormantos massif and of Toledo, Salamanca

and Zamora suggest heterozygous paracentric and pericentric chromosome inversions (6).

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Appendix

Table 6 Summary of karyotype of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	*N*	LBC		LBL		LTC		A_1 (range)	CV (%)	A_2 (range)	CV (%)
		Mean ± SD (range) (μm)	CV (%)	Mean ± SD (range) (μm)	CV (%)	Mean ± SD (range) (μm)	CV (%)				
ROS	150	2.18 ± 0.43 (1.03–4.18)	19.72	2.91 ± 0.60 (1.87–6.91)	20.53	5.11 ± 0.86 (3.60–8.61)	16.92	0.28 ± 0.03 (0.20–0.39)	12.39	0.13 ± 0.08 (0.08–1.00)	59.13
OBL	75	1.86 ± 0.37 (0.45–2.81)	19.89	2.51 ± 0.40 (1.71–3.47)	15.97	4.37 ± 0.51 (3.21–6.14)	11.63	0.27 ± 0.03 (0.19–0.32)	11.97	0.14 ± 0.03 (0.10–0.21)	22.84
SEM	90	2.32 ± 0.50 (1.13–3.52)	21.55	3.11 ± 0.47 (2.15–5.25)	15.16	5.41 ± 0.77 (3.83–7.79)	14.22	0.27 ± 0.03 (0.21–0.32)	9.45	0.15 ± 0.04 (0.10–0.21)	24.25
MEL	24	2.64 ± 0.56 (2.06–4.53)	21.21	3.51 ± 0.68 (2.68–5.30)	19.35	6.14 ± 1.13 (4.74–9.83)	18.37	0.30 ± 0.04 (0.22–0.36)	12.83	0.14 ± 0.03 (0.10–0.19)	20.08
CAN	105	2.13 ± 0.47 (0.59–3.71)	22.06	2.91 ± 0.53 (2.09–4.52)	18.21	5.04 ± 0.82 (3.72–8.14)	16.25	0.28 ± 0.03 (0.20–0.35)	11.97	0.12 ± 0.03 (0.09–0.21)	21.93
MIX											
ROS	18	2.50 ± 0.43 (1.67–3.31)	17.20	3.27 ± 0.63 (2.30–5.93)	19.19	5.76 ± 0.82 (4.43–9.23)	14.29	0.28 ± 0.03 (0.23–0.31)	9.45	0.11 ± 0.02 (0.09–0.16)	14.01
OBL	19	2.19 ± 0.32 (1.55–2.99)	14.61	2.98 ± 0.41 (2.24–3.69)	13.72	5.18 ± 0.56 (4.26–6.43)	10.84	0.28 ± 0.03 (0.25–0.32)	10.27	0.13 ± 0.02 (0.10–0.16)	18.37
HYB	38	2.17 ± 0.57 (1.20–3.10)	26.26	3.06 ± 0.51 (2.28–4.28)	16.50	5.23 ± 0.68 (4.28–6.49)	12.91	0.27 ± 0.04 (0.21–0.30)	13.26	0.11 ± 0.01 (0.10–0.13)	12.60

N Number of metaphase plates studied, *LBL* mean length of the long arm, *LBC* mean length of the short arm, *LTC* mean of the total length of the chromosome, *CV* coefficient of variation, A_1 intrachromosomal asymmetry index, A_2 interchromosomal asymmetry index, *SD* standard deviation

Table 7 Summary of meiotic configuration frequency of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	2*n*	*N*	I	OII	CII	OIII	CIII	OIV	CIV	CV	CVI	CVIII	CIX	B	Frag
ROS-CN	2×	151	–	5.12	3.88	–	–	–	–	–	–	–	–	–	–
ROS-T	2×	201	0.007	3.41	4.88	–	–	0.30	0.36	–	0.04	–	–	0.20	–
ROS-T	4×	10	–	9.90	7.45	–	–	–	0.65	–	–	–	–	0.55	–
OBL	2×	93	–	5.88	3.12	–	–	–	–	–	–	–	–	–	–
SEM	2×	93	–	4.70	3.50	–	–	0.40	0.40	–	–	–	–	0.32	–
MEL	2×	46	–	4.75	3.80	–	–	0.25	0.20	–	–	–	–	0.26	–
CAN	2×	82	–	3.74	4.66	–	0.09	0.21	0.30	–	–	–	–	–	–
CAN	4×	10	–	8.42	8.42	–	–	–	1.16	–	–	–	–	0.29	–
MIX															
ROS	2×	257	0.15	3.70	4.23	–	0.08	0.15	0.31	–	0.15	0.18	0.05	0.20	0.02

Table 7 continued

Taxon	2*n*	*N*	I	OII	CII	OIII	CIII	OIV	CIV	CV	CVI	CVIII	CIX	B	Frag
OBL	2×	76	–	4.54	3.88	–	–	0.11	0.40	–	0.07	–	–	0.30	–
HYB	2×	107	0.07	4.24	4.27	0.03	0.07	0.07	0.12	0.007	0.05	0.06	0.02	0.17	–

CN Populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif, Álava, Ávila and Burgos provinces, *T* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora, *N* number of meiocytes studied, *B* B chromosomes, *I* univalent, *OII* bivalent ring, *CII* rod bivalent, *OIII* trivalent ring, *CIII* trivalent chain, *OIV* quadrivalent ring, *CIV* quadrivalent chain, *CV* pentavalent chain, *CVI* hexavalent chain, *CVIII* octovalent chain, *CIX* nonevalent chain, *Frag* fragment

Table 8 Summary of chiasma frequency of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	*N*	QFT			QFM			QFI		
		Range	Mean ± SD	CV (%)	Range	Mean ± SD	CV (%)	Range	Mean ± SD	CV (%)
ROS-CN	151	8–20	15.22 ± 2.49	16.36	3–18	11.19 ± 3.34	29.84	0–9	2.76 ± 1.97	71.37
ROS-T	201	9–18	13.62 ± 2.19	16.07	5–16	11.09 ± 2.60	23.44	0–6	2.52 ± 1.56	61.90
OBL	93	11–18	14.88 ± 1.74	11.69	6–18	12.35 ± 2.68	21.70	0–6	2.53 ± 1.70	67.19
SEM	93	10–18	14.14 ± 2.32	16.41	1–17	11.77 ± 2.83	24.04	0–17	2.26 ± 1.67	73.89
MEL	46	10–18	14.04 ± 2.01	14.32	5–16	11.22 ± 2.72	24.24	0–6	2.83 ± 1.76	62.19
CAN	82	9–26	13.35 ± 3.56	26.66	5–18	9.15 ± 2.76	30.16	0–10	4.21 ± 2.21	52.49
MIX										
ROS	257	9–18	13.89 ± 2.13	15.33	4–19	11.16 ± 2.73	24.46	0–9	2.82 ± 1.95	69.14
OBL	76	10–18	13.99 ± 2.08	14.87	6–17	11.53 ± 2.94	25.49	0–6	2.72 ± 1.75	64.34
HYB	107	9–18	13.97 ± 2.11	15.10	5–18	11.15 ± 2.68	24.03	0–6	2.82 ± 1.47	52.13

CN Populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif, Álava, Ávila and Burgos provinces, *T* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora, *N* number of individuals studied, *QFT* total chiasma frequency, *QFM* terminal chiasma frequency, *QFI* interstitial chiasma frequency, *CV* coefficient of variation, *SD* standard deviation

Table 9 Proportion (%) of meiocytes displaying abnormal anaphase in *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	*N*	Delayed disjunction of the homologous and non-homologous chromosomes	Simple chromosome bridge	Double chromosome bridge
ROS-CN	151	–	–	–
ROS-T	201	25.37 (51)	22.39 (45)	14.93 (30)
OBL	93	–	–	–
SEM	93	48.39 (45)	54.84 (51)	3.23 (3)
MEL	46	19.57 (9)	26.09 (12)	–
CAN	82	21.95 (18)	29.27 (24)	–
MIX				
ROS	257	25.68 (66)	35.02 (90)	21.01 (54)
OBL	76	51.32 (39)	55.26 (42)	31.58 (24)
HYB	107	33.64 (36)	44.86 (48)	30.84 (33)

The number of meiocytes with abnormal meiosis is shown *in parenthesis*

N Total number of meiocytes studied, *CN* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif, Álava, Ávila and Burgos provinces, *T* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora provinces

Table 10 Variability of the PCA factors of the meiotic characteristic by means of nested MANOVA aimed at detecting variation between and within taxa and within the hybrid complex. Variance components are shown in parenthesis

Source of variation	dfe, dfr	Factor1	Factor2	Factor3	Factor4
Variation between and within taxa					
BTX	4, 450	12.57 (20.12)***	21.33 (9.70)***	3.46 (2.80)*	13.11 (1.50)***
BPT	14, 450	15.59 (23.00)***	7.06 (14.00)***	4.32 (8.40)**	10.03 (16.00)***
BITP	197, 450	2.03 (19.30)***	0.84 ns	1.10 ns	1.78 (16.80)***
Variation within the hybrid complex					
PB	5, 299	8.79 (9.10)***	2.86 (2.10)*	16.07 (16.40)***	14.33 (12.50)***
BTP	7, 299	4.46 (10.40)**	1.54 ns	0.23 ns	1.47 ns
BIPT	128, 299	1.41 (2.80)*	0.97 ns	1.70 (15.40)**	2.04 (22.00)***

BTX Between taxa, *BPT* between populations within each taxon, *BITP* between individuals in each taxon and population, *PB* between populations, *BTP* between taxa within each population, *BIPT* between individuals in each population and taxon, *dfe* degree of freedom of the effect, *dfr* degree of freedom of the error

*$P < 0.05$, **$P < 0.001$,***$P < 0.0001$, *ns* $P > 0.05$

Table 11 Summary of pollen stainability of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS), *S. oblongifolia* (OBL), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. canescens* (CAN) and in the hybrid complex (MIX) of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (HYB)

Taxon	*N*	Range (%)	Mean ± SD (%)	CV (%)
ROS-CN	151	39–100	94.68 ± 12.88	13.60
ROS-T	201	9–100	92.07 ± 15.99	17.36
OBL	93	60–100	91.14 ± 8.13	8.90
SEM	93	4–100	86.89 ± 13.91	16.01
MEL	46	66–100	92.37 ± 6.46	6.99
CAN	82	50–100	94.43 ± 7.84	8.30
MIX				
ROS	257	5–100	84.47 ± 25.20	29.83
OBL	76	12–100	82.35 ± 21.51	26.12
HYB	107	11–100	65.29 ± 30.67	46.97

N Total number of anthers studied, *CN* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of the Guadarrama massif, Álava, Ávila and Burgos provinces, *T* populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca and Zamora provinces, *CV* coefficient of variation, *SD* standard deviation

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