A review of *Sciurus Group* studies on the red squirrel (*Sciurus vulgaris*): presence, population density and colour phases in Lombardy (Italy)

Carlo M. Biancardi*, Claudio G. Gnoli

Abstract - During the nineties of the last Century, under the inspiration of Luigi Cagnolaro, it had been founded a "Sciurus group", within the Research Centres of the Società Italiana di Scienze Naturali. Aim of the group was to start a campaign of researches on red squirrel populations in Lombardy, Italy promoting field master thesis on various topics: colour phases and indirect estimations of population densities. The researches were carried out during 10 years in 5 study areas. An investigation and census with questionnaires were started in parallel, with the collaboration of Forest Guards (CFS), Ecological Guards (GEV) and students of biological and natural sciences. The results were published in Italian or exposed in national and international congresses. This review has the purpose to show and critically discuss the overall results, and make them available to a broader audience. The dark colour phase, in the red squirrel, resulted associated to elevation and conifer woodlands, according to the thermoregulation theory, which consider the dark fur thicker and more suitable in cold environments. Squirrel population densities are subject to periodical fluctuations, in conifer woodlands, due to cones availability. Cone crops are subject to cycle with years of great production followed by years of lean. The recorded squirrel densities are middle-lows, but in line with data from similar environments.

Key words: Red squirrel, *Sciurus vulgaris*, Nest density, Colour phases.

Riassunto - Revisione del gruppo di lavoro "*Sciurus group*" sullo scoiattolo commune (*Sciurus vulgaris*): presenza, densità di popolazione e fasi di colore.

Negli anni '90, su impulso di Luigi Cagnolaro, è stato fondato un gruppo di lavoro "Sciurus group", all'interno dei Centri Studi della Società Italiana di Scienze Naturali. Scopo del gruppo era avviare ricerche sulle popolazioni di scoiattolo comune europeo in Lombardia, promuovendo anche tesi di laurea di campo su vari aspetti, dalle fasi di colore alla stima indiretta delle densità di popolazione. Le ricerche sono proseguite circa 10 anni, e hanno interessato 5 aree di studio. Parallelamente è stata avviata un'indagine e un censimento delle fasi di colore con questionari, dapprima presso le stazioni del Corpo Forestale dello Stato, e successivamente allargata a Guardie Ecologiche Volontarie e studenti di Scienze Biologiche o Naturali.

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Received: 21 March 2016. Accepted for publication: 10 June 2016. I risultati delle singole ricerche furono esposti a convegni e congressi o pubblicati in italiano. Questo lavoro di review ha lo scopo di riprendere e attualizzare i risultati complessivi, discuterli e renderli disponibili a un più vasto pubblico di ricercatori. La fase di colore scuro, nello scoiattolo, è risultata associata ad altitudini più elevate e a boschi con prevalenza di conifere, in accordo con la teoria della termoregolazione, che ritiene la pelliccia scura più folta e adatta ad ambienti freddi. Le popolazioni di scoiattolo comune sono soggette, specialmente in ambienti di conifere, a fluttuazioni delle densità di popolazione legate alla disponibilità di coni (e quindi semi), anch'essa soggetta a cicli con anni di produzione abbondante (pasciona) ed altri di magra. Le densità di popolazione rilevate sono medio-basse, ma in linea con le tipologie ambientali riscontrate.

Parole chiave: Scoiattolo, *Sciurus vulgaris*, Fluttuazione delle densità di popolazione, fasi di colore.

INTRODUCTION

The "Sciurus group" was a research unit, founded in the 1990s by Luigi Cagnolaro within the Study Centres of the Societá Italiana di Scienze Naturali, in collaboration with the Vertebrate Zoology section of the Museum of Natural History of Milan. The direction of the unit was assigned to the authors of this review, and based on voluntary work.

During the period 1991-2000 different studies, most of them master thesis, have been carried out under the co-tutorship of Luigi Cagnolaro and/or Carlo M. Biancardi, Claudio Gnoli and Laura Rinetti (Fig. 1). The results of these researches were presented to national and international congresses and symposia, and partially published, in Italian, on local scientific journals or bulletins. The purpose of this work is to take the highlights of the scientific production of that cycle, critically review the main results and make them available to a wider international audience.

The Eurasian red squirrel (*Sciurus vulgaris* L., 1758) populations of Italy and the UK are threatened by the introduction of the alien invasive grey squirrel (*Sciurus carolinensis* Gmelin, 1788) (*e.g.* Bertolino *et al.*, 2014). At the beginning of the nineties, the "known" dangerous situations in Italy were in Piedmont and Genoa Nervi, while in Lombardy we feared the possible expansion of grey squirrels from the west.

One of the greatest concerns was about habitat fragmentation of lowland forests (*e.g.* Celada *et al.*, 1994).



However, the status of the red squirrel populations in the mountain part of the region was little known and, indeed, mountain conifer woodlands were considered safer for red squirrels, with respect to the alien grey squirrel invasion (*e.g.* Gurnell, 1987).

Following the huge work made by Wauters & Dhondt (1988; 1990), and the data from the (then) recent works of Grodzinski & Sawicka-Kapusta (1970), Purroy & Rey (1974), Grönwall (1982), we decided for low-budget but extended researches using indirect methods for estimating the squirrel density. In parallel, we started an investigation on the relative frequencies of different colour-phases, and their relationship with ecological parameters such as elevation and forest composition. The red squirrel is characterized by a coat colour polymorphism, which can be more or less evident across the different subspecies (Fig. 2). The S.v. fuscoater Altum, 1876, the subspecies present in Northern Italy, can show a high degree of polymorphism. The colour change can be gradual, as shown in Fig. 2, but usually it is possible to discriminate two (red and dark) or three (red, brown and black) colour phases (Andera, 1985; Wauters et al., 2004). When the census is made by visual survey, it is easier to discriminate only between red and dark colours, especially if the animals are spotted in woodlands, at distance, with the help of binoculars.

This review is based on data presented at the *II Congresso Italiano di Teriologia* (Biancardi, 1998a; 1998b; Biancardi et al., 1998), to the 6th Squirrel Workshop (Biancardi & Gnoli, 2001), and partially published in Biancardi (1999) and Aristarchi & Canu (1999). The master theses produced during that cycle of researches were that of: Alessandra Speranza (1992), Claudio Aristarchi (1996), Franco Dell'Acqua (1998), Andrea Viganó (1998), Lorena Aquilini (2000).

MATERIALS AND METHODS

Presence and colour phases

The use of questionnaires to collect information about animal populations is a rapid and cost effective methodology, which today lies under the definition "citizen science" (Dickinson *et al.*, 2010). The method has been used in the past century to investigate on presence and colour phases of red squirrel populations (Zawidzka, 1958; Andera, 1985), and nowadays replaced by more modern and effective methodology (*e.g.* Mori & Menchetti, 2014). For the reasons exposed in introduction, we worked with two colour phases.



Fig. 1 - A moment of the fieldwork in Luinese. From the left: Gianni Schiroli, Andrea Viganò, Luigi Cagnolaro e Laura Rinetti. (Photo by Carlo Biancardi).



Fig. 2 - Fur colour polymorphism in *Sciurus vulgaris*. Collection of the Museum of Natural History of Milan MSNM. (Photo by Carlo Biancardi).

Questionnaires (n=132), prepared by the authors and distributed at the end of 1991, were filled by members of the *Corpo Forestale dello Stato* (CFS, Forest Rangers) of Lombardy (Northern Italy). They included 8 questions, concerning the general presence and abundance of the species and data from punctual observations with i) the colour phase: dark or red; ii) the altitude and iii) the environment. The latter part of the questionnaire, concerning the observation of individuals, was used to collect information from *Guardie Ecologiche Volontarie* (GEV, Ecological Guards) and from the students during their surveys.

Population ecology

The field researches were carried out in five study areas, characterised by different kind of woods (Fig. 3; Tab. 1). Here, the population densities of red squirrels were estimated using two indirect methods (Sutherland, 2006; Gurnell *et al.*, 2009):

i) the feeding sign survey by quadrat sampling of 5×5 m.

ii) the drey counts by quadrat sampling of 1 ha $(100 \times 100 \text{ m})$ or on line transects. The number of hectares and the length of the linear transects are reported in Tab. 1.

Feeding sign survey

This technique is applicable in coniferous woods, where the cones eaten by squirrels remain on the ground

and are easily detected and identified (*e.g.* Bang *et al.*, 2001). The study area was divided in quadrats of 5×5 m, and the sampling quadrats were chosen using a random

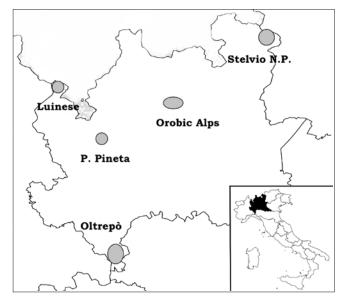


Fig. 3 - Study areas in Lombardy. Box: geographic position of Lombardy (black) in Italy.

Author	Study area	Macro-area	Forest composition	Feeding remains (cones)	Dreys: quadrat sampling (Ha)	Dreys: transect sampling (km)
Speranza, 1992	Piazzatorre (BG)	Orobic Alps	Conifers (Spruce, Silver fir)		11	
Speranza, 1992	San Lucio (BG)	Orobic Alps	Conifers (Spruce)		12	
Aristarchi, 1996	Uzza (SO)	Stelvio N.P.	Conifers (Larch)		50	
Dell'Acqua, 1998	Appiano Gentile (CO), Tradate (VA)	Parco Pineta	Conifers and Broadleaved (Scots pine, Oaks)	Х		26
Viganò, 1998	Luinese (VA)	Prealps	Mixed Broadleaved (Oaks, Beech, Chestnut)			24
Aquilini, 2000	Valle Staffora (PV)	Oltrepò	Broadleaved (Oaks)			19

Tab. 1 - Study areas, forest composition, main tree species and census methods employed.

integer generator. During the delimitation of the sampling quadrats (first visit, t0), all the cones were removed. Thereafter, at periodic intervals (t1, t2,...), the new cones were collected and removed from the sampling area. In this way, the estimated number of cones eaten by squirrels per unit area and per day can be calculated. The average number of cones eaten per day by one red squirrel can be estimated from: i) the energetic requirement of the species: an adult red squirrel was supposed to need between 330 and 420 kJ/day (Grönwall, 1982), but more recent data set this energy requirement between 400 and 700 kJ per day (Gurnell *et al.*, 2009); ii) the amount of energy of an unit mass of seeds (kJ/g); the average number of seeds, or their weight, per cone.

Drey counts

The density of red squirrel dreys is a proxy of the population density of the species (Wauters & Dhondt, 1988; 1990). Contrary to what is commonly thought, red squirrels infrequently build their nest in holes in trees, called dens, but more commonly they built nesting structures, called dreys, in the tree canopy. Dreys are sub-spheric structures of 30-50 cm, made by twigs and leaves, usually built close to the trunk or one of the main branch of a tree (Biancardi & Do Linh San, 2006). A red squirrel can build and maintain a variable number of dreys within its territory, usually between 4 and 6 (Wauters & Dhondt, 1990). Dreys can be more easily detected in broadleaved woodlands in winter, when the leaves are gone (Gurnell et al., 2009). The same happens in larch woods (Aristarchi, 1996). In other conifer woods the count, either on quadrats or on line transects, takes more time and the possibility of underestimate the real number of dreys is higher.

The study area was divided in quadrats of 100×100 m and, like for feeding sign survey, the sampling quadrats were chosen using a random integer generator.

The following additional data were collected during the surveys: position of the drey (close to the trunk or different), orientation, height, tree species and tree diameter (at 1.5 m from the ground).

Statistical analysis

The differences in colour phase ratio and the distributions of observed against expected frequencies were tested using Chi-squared or G-test of goodness-of-fit (So-kal & Rohlf, 2003). The orientation of squirrel dreys on the tree trunks was analysed applying the Rayleigh *z*-test, with the null hypothesis (H_0) that there is no preferred direction (Batschelet, 1981).

RESULTS

Presence and colour phases

The feedback from CFS stations was geographically inhomogeneous: half of the information came from the district of Brescia (BS), one third from the districts of Bergamo (BG) and Sondrio (SO), and the rest (<20%) from the districts of Como (CO), which included the current territory of Lecco, Varese (VA), Pavia (PV) and Milano (MI), which included the current territory of Monza-Brianza. The Centro Studi did not get reply from the districts of Mantova (MN) and Cremona (CR).

A total of 1346 observations of red squirrels were recorded, and the ratio between the dark and red colour phases (CPR) resulted 2:1. The CPR significantly changed in favour of the dark phase while ascending in elevation (G=48.56, df=5, P<0.01) (Fig. 4). Slight differences of CPR in favour of the dark phase occurred as well in all the conifer woodlands, while an opposite trend was observed in broadleaved woodlands (regardless of the altitude).

Feeding sign survey

The feeding sign surveys were carried out in two study areas (Tab. 1). In one case the sampling quadrats were built in a scots pines wood, in the second area the

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1600 - 2000 m
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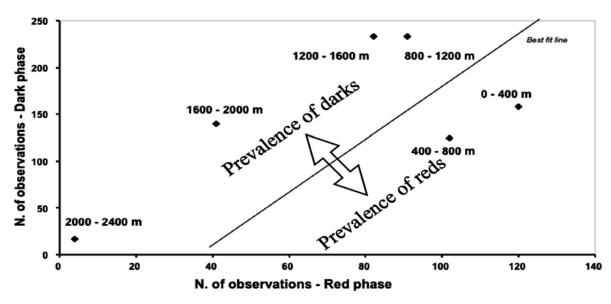


Fig. 4 - Observations of red and dark phases per altitudinal range. The dark phase became prevalent, with respect to the mean colour phase ratio, above 800 m a.s.l.

dominant species was the Austrian pine (*Pinus nigra*). The estimated numbers of scots pine cones eaten by one red squirrel per day was taken from literature: Purroy & Rey (1974) estimated this number being between 114 and 143. The energy content of Austrian pine seed is 15.5 kJ/g (Grodzinski & Sawicka-Kapusta, 1970). Aquilini (2000) calculated the average weight of the seeds contained in one cone: 0.34 ± 0.3 g/cone. The estimated number of Austrian pine cones eaten per day (62-80) was based on the energetic requirements data in Grönwall (1982). The new estimation, based on a requirement of 400-700 kJ/day (Gurnell *et al.*, 2009), set this number in the range of 83-146 cones per days per squirrel. The estimated densities of population from feeding sign survey are reported in Tab. 2.

Drey counts

A total number of 1179 dreys were counted during the whole period in all the study areas (Tab. 3). Southern exposures (South-Southeast-South-Southwest) were generally preferred (Rayleigh z-test; P<0.05 in all areas; P<0.001 on the total mean), but in mountain areas the results can be influenced by the slope exposure (Fig. 5). The dreys resulted mainly built between the trunk and one main branch (Chi-square: P<0.001), and in the higher part of the canopy: the ratio between the height of the nest and of the tree was 0.816 ± 0.117 . Two abandoned dreys, in Uzza and Luinese, have been found fallen on the ground, collected and analysed (Tab. 4).

The drey densities, together with an estimation of the red squirrel population density, as found in quadrat sampling and in linear transect counts are reported in Tab. 5.

During their survey, Aristarchi & Canu (1999) collected 208 mushrooms stored by squirrels on trees. Tab. 2 - Average density of population estimated from feeding sign surveys.

¹According to the average number of cones eaten per day (Purroy & Rey, 1974); ²According to the energy values in Gurnell *et al.* (2009).

Study area	Years	Average squirrels/Ha
Parco Pineta ¹	1995-97	1.4-1.8
Monte Alpe ²	1998-99	0.4 0.7

Tab. 3 - Overall number of dreys.

Type of wood	N. of dreys	
Mixed broadleaved	265	
Larch woods	252	
Mixed broadleaf + conifers	245	
Chestnut woods	171	
Chestnut + other broadleaf	130	
Mixed conifers	89	
Ash and alder	5	
Black locust	8	
Beech woods	7	
Shrubs	3	
Moor with trees	2	
Downy oak woods	1	
Isolated tree	1	

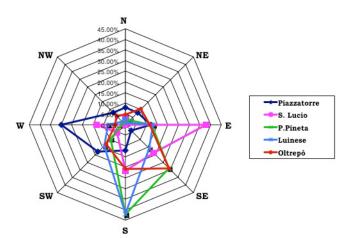


Fig. 5 - Orientation of the red squirrel dreys per study area.

Analysis of a drey	Stelvio	Luinese
Drey measures	32 × 30 × 12 cm	40 × 19 × 13 cm
Total weight	666 g	210 g
Frame weight	334 g	100 g
Stuffing weight	332 g	110 g
N. of twigs		64
Max. diameter of the twigs		5 mm
Lenght of the twigs		10-40 cm
Ramifications of the twigs		0-4

Tab. 4 - Analysis of two fallen dreys

DISCUSSION

This review covers a time span of about ten years, till the beginning of the 2000s. Some information are of course out-dated, *i.e.* the estimations of population densities, but could be used as historical reference of the status of the red squirrel populations at that time in these areas.

There is an old debate about the adaptive meaning of the coat colour polymorphism in some sub-species of Sciurus vulgaris, like the S.v. fuscoater. In particular Voipio (1970) claimed the role of the mean temperatures on the colour polymorphism. According to Voipio & Hissa (1970) the melanic individuals would have thicker furs and longer hairs with respect to brown and reddish. The greater insulating power of melanic furs would favour a better thermoregulation in cold environments. The same idea was suggested by Hancock & Nash (1979, in Gurnell, 1987), for Sciurus alberti. The significant relationship between dark colour phase and higher altitudes in mountains found during the researches of the "Sciurus group" is in agreement with the thermoregulation theory. More recently, a huge work involving 9 study areas has been carried out by Wauters et al. (2004), considering the three colour phases red, brown and black. According to the authors, better thermoregulation and more cryptic coat would give an advantage to melanic individuals in cold and dense spruce-fir forests of the Alps. Concealment has also been recently indicated as the factor more strongly related to the coat colour of Sciuromorpha (Ancillotto & Mori, 2016). However, a differential survival rate of the morphotypes, associated to temperature/elevation or predation has not been demonstrated (Wauters et al., 2004).

The feeding sign survey in conifer woodlands is one of the monitoring strategies that can be used when only one species of squirrels is likely to be present (Gurnell

Tab. 5 - Estimated dreys and red squirrel densities. Pre-br) average pre-breeding density; Year) average of the whole year. *) estimation based on mean values of 4.85 dreys/squirrel in broadleaved woodlands and 5.17 dreys per squirrel in conifer woodlands (Wauters & Dhondt, 1990).

Study area	Method	Dreys/Ha (min-max)	Sq/Ha*
P. Pineta	Line transects	2.1 (year)	0.4
Luinese '94	Line transects	1.0 (pre br.)	0.2
Luinese '95	Line transects	1.0 (pre br.)	0.2
Luinese '97	Line transects	0.4 (pre br.)	0.1
Val Staffora	Line transects	0.8 (pre br.)	0.2
S. Lucio	Quadrat sampling	3.4 (year)	0.7
Piazzatorre	Quadrat sampling	4.1 (3.1-4.7)	0.8 (0.6-0.9)
Uzza ('93)	Quadrat sampling	4.5 (pre-br.)	0.9
Uzza ('94)	Quadrat sampling	5.0 (pre br.)	1.0
M.te Alpe	Quadrat sampling	1.8 (1.7-2.0)	0.3 (0.3-0.4)

et al., 2009). Indeed, at the time of this cycle of studies, introductions of grey squirrels in Lombardy were not known (Martinoli et al., 2010). The power of this method to estimate population densities is moderate, however better than drey counts (poor-moderate) and better than others indirect methods, out of visual survey (Gurnell et al., 2009). One of the critical points of the estimation is that there are different approximations: the mean number of seed per cone, the mean energy of a cone, assuming that a high percentage or all the seeds are eaten, the energy requirement of an individual. There is also the possibility that squirrels are taking advantage of other feeding sources, like mushrooms, which can be available in conifer woodlands as well (Lurz & South, 1998; Aristarchi & Canu, 1999). The densities estimated with this method can dramatically change according to the annual cone crop, but this reflects the real fluctuations of population density related to food availability (Gurnell et al., 2004).

The results of drey counts have been obtained using the average number of dreys per squirrel as reported in Wauters & Dhondt (1988; 1990). To our knowledge, those are the most recent and still used available estimations (Gurnell *et al.*, 2004; 2009).

Drey counts, like feeding sign survey, cannot discriminate between red and grey squirrels (Gurnell, 2004). This is one of the reasons why this monitoring method has fallen into abeyance in Italy, and replaced by hairtube surveys (Bertolino *et al.*, 2009). More recently, also camera trapping has been proved to be an efficient method for assessing the presence and monitoring the populations of arboreal small mammals (Di Cerbo & Biancardi, 2013).

The ecological characteristics of the dreys exposed in results are in line with other published data (*e.g.* Fornasari *et al.*, 1997). Red squirrels prefer to build their nests in a sheltered and protected position, near the main trunk and in the medium-higher part of the canopy, but with an orientation that can favour a better insolation.

In conclusion, the cycle of researches promoted by Luigi Cagnolaro in the nineties can still represent a historical point of reference to understand and deal with a situation that, with the senseless introductions of the grey squirrel in Lombardy, has dramatically changed. The investigated areas, being still out or partially interested by the presence of the grey squirrel, can be part of a pool of red squirrel populations suitable for a natural expansion after a desirable eradication of the alien species.

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