Advances in Research on Ecophysiology and Evolutionary Ecology: The Red-Legged Partridge as a Study Model

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Introduction: From Game Species to Evolutionary Ecology Starlet

Most scientific research on the red-legged partridge produced during the twentieth century was focused on the basic breeding biology, spatial ecology or sanitary aspects of this species (see Chaps. "Habitat Use and Selection: Is Habitat Management the Key to Restore Red-Legged Partridge Populations?" and "Health Monitoring and Disease Control in Red-Legged Partridges"). In many cases these studies were purely descriptive and closely related to game management. Nevertheless, with the advent of the new century, a relevant shift occurred, with a series of studies where the red-legged partridge was used to address relevant aspects of evolutionary biology and in particular of the sub-discipline of evolutionary ecology. This field of research explores how evolution shaped the genotype and phenotype of the species, as well as the way they interact with the environment and with other organisms. Nowadays, the red-legged partridge is increasingly recognized as a useful model system for addressing burning questions in evolutionary ecology, which at the same time is contributing to unravel some aspects of its behaviour, ecology and physiology that remained ignored.

There are several intrinsic features that make the red-legged partridge an interesting model for research in evolutionary ecology and ecophysiology. It is a socially monogamous and slightly sexually dimorphic species, and both sexes collaborate in clutch and brood attendance, although offspring care and breeding investment is higher in females (Cramp and Simmons 1980). Also, significant rates of extra-pair paternity and intraspecific brood parasitism have been described (Casas et al.

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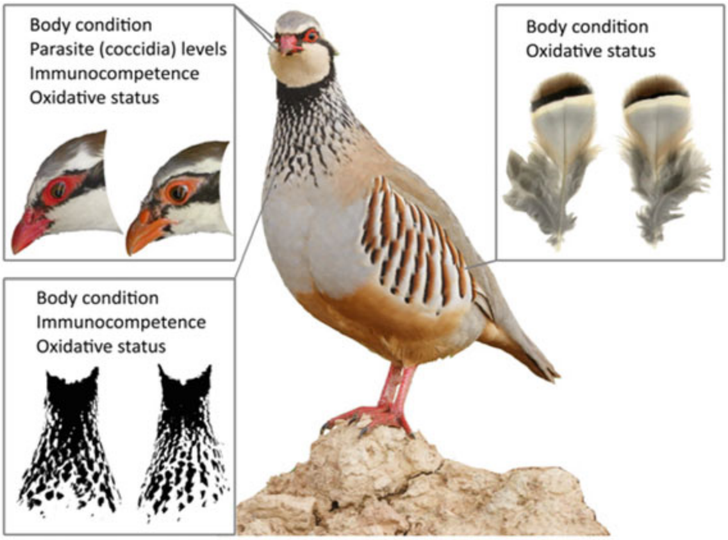

Fig. 1 Ornaments of the red-legged partridge and their relationship with quality-related traits. Boxes contain illustrative images of the variability of each character and the specific quality-related traits for which a connection has been empirically established. *Carotenoid-based colouration*: beak and eye ring redness are considered together because they tend to respond to the same sources of variation, although their responsiveness and dynamism differ (higher for the eye ring). The informative potential of leg colouration has not been addressed yet, although its biochemical basis is similar to that described for eye ring and beak. *Black bib*: the box contains two bibs with similar eumelanin-pigmented areas but whose patterns exhibit high (left) and low (right) fractal dimensions; the area covered by the trait is related to the oxidative status during moult, whereas the fractal dimension is related to the body condition and immunocompetence of the individual. *Barred pattern of the flanks*: the size of the black band (specifically, of the black area of the ventral portion of each feather) is related to the body condition of the individual, whereas the size of the brown and black bands is indicative of the oxidative status during feather growth. Central picture: José Ardáiz. Boxes' pictures: Lorenzo Pérez-Rodríguez

2006a, b), indicating that the sexual conflict within a pair remains even though the social pair bond is maintained. Such breeding system is somehow representative of the vast majority of bird species (Ligon 1999). The red-legged partridge is also a good model species for studying the function and evolution of ornamental pigmentation, as it displays two of the most widespread colourations among animals: carotenoid and melanin-based traits (Fig. 1). Interestingly, these colourations, besides their likely role as social signals of quality, are not displayed in a conspicuous or flashy fashion. Instead, the redness of the beak, eye rings and tarsi and the shape and size of the flank bands and black bib can only be fully perceived at a short distance. From medium and long distances, birds remain mostly cryptic thanks to the mimetic olive dorsal plumage, which is particularly useful against aerial predators that rely on visual cues to detect their prey. Therefore, the colour ornaments of the red-legged partridge seem to have evolved as a product of the compromise between signalling and predation avoidance. When studying the evolution of life-history and phenotypic traits, extreme cases (e.g. strictly polygamous species, lek mating systems, highly conspicuous or bulky ornaments) are often preferred as models because the trade-offs show up more easily. However, most avian species are far from these extreme strategies, having evolved intermediate strategies as a result of environmental and evolutionary constraints. Study systems like the red-legged partridge are therefore required to get a more representative, generalizable, balanced and unbiased view of adaptive and evolutionary processes.

The red-legged partridge shows some clear phenotypic (e.g. carotenoid-based teguments and melanin-based patterns in the bib and flanks, slight sexual dimorphism) and behavioural (e.g. socially monogamous pair bonding, biparental care) parallelisms with the unquestionable major avian model system for evolutionary biology studies in captivity, the zebra finch (*Taeniopygia guttata*) (Griffith and Buchanan 2010). The vast knowledge on the zebra finch biology and genome as well as simple practical issues like its handy small size and perfect adaption to captive conditions results in an unparalleled potential for captivity experiments with no need of large infrastructures. However, the red-legged partridge possesses some characteristics that, from a logistic perspective, are useful for designing experiments. For instance, partridges are relatively large birds. Although this might impose some space requirements for housing, it also presents some advantages, for example, the possibility to collect higher volumes of blood for different analyses and the use of radio-tagging techniques for field studies. The large number and size of eggs laid by females enable manipulative studies of maternal effects, and the precocial nature of the species allows isolating pre- and postnatal parental effects following simple farming procedures. In fact, partridge farming has led to the development of useful housing and rearing protocols and to the commercial availability of specific equipment. Finally, unlike other bird species commonly used as captive study systems in evolutionary biology —Japanese quail (*Coturnix japonica*), common pheasant (*Phasianus colchicus*), zebra finch— the red-legged partridge is an autochthonous species in most of the geographic areas where it is commercially bred. This is a great opportunity for scientists to combine studies in captivity and wild conditions while settled in the same geographic location.

Most research on evolutionary ecology using this species as a model has focused on the function of carotenoid and melanin-based ornamental colourations, with a particular emphasis in the physiological mechanisms regulating their expression. Below I will summarize some of the main results from these studies. Also, I will identify key questions and unexplored areas that warrant special attention of the research community in the near future.

The Redness of the Red-Legged Partridge as a Study System of Carotenoid-Based Ornamentation

The red colourations of the beak, eye ring and tarsi of the red-legged partridge are probably the most distinct and characteristic traits of this species, as highlighted by its Latin name as well as by its common name in most languages (although, ironically, its "red legs" remain poorly studied as compared to its other two red traits). This kind of showy traits usually plays a role in signalling individual quality during mating or in social interactions in many taxa but particularly in birds (Hill and McGraw 2006; McGraw 2006a). A direct and experimental assessment of the role of these traits in mate choice and intra-sexual interactions in the red-legged partridge is still pending, but there are some indirect evidences supporting such a signalling role. For instance, experiments in captivity have shown that females paired with males whose eye rings and beaks were artificially intensified by means of red paint tended to start laying eggs earlier and produced significantly a higher number of eggs than females mated to un-manipulated males (Alonso-Álvarez et al. 2012). Also, females mated with these "made-up" males varied the allocation of key components of the egg yolk (carotenoids, androgens) as compared to control females. These results indicate that females actually use the redness of their mates as cue for key reproductive decisions, as expected from traits used as signals of quality in sexual selection processes. Another set of indirect evidences of the signalling value of these traits comes from field studies reporting an assortative mating based on beak redness in this species, a pattern that would arise from a reciprocal mate choice based on this character (Casas et al. 2010).

The characteristic red colouration of the beak, eye rings and legs of the red-legged partridge results from the accumulation of carotenoids (Pérez-Rodríguez and Viñuela 2008; García-de Blas et al. 2011, 2013). Carotenoid pigments are a series of large lipophilic compounds produced by photosynthetic organisms and certain bacteria and fungi (Goodwin 1984). Partridges, like the rest of animals, cannot synthesize carotenoids de novo but have to obtain them from their diet (Goodwin 1984). Importantly, this colour production mechanism is not exclusive at all of the red-legged partridge: carotenoid-based colouration is widespread in the animal kingdom, resulting in yellow, orange or red striking visual displays in many fishes, amphibians, reptiles and birds. During the last decades, evolutionary and behavioural ecologists have become increasingly interested in these traits because they often play key roles in communication, being used as signals of bearer quality in social and mate choice contexts (Goodwin 1984; Hill 2002; McGraw 2006a; Pérez-Rodríguez et al. 2013a, b). Theory predicts that signal expression must be linked to the intrinsic characteristics of the bearer, implying that signal development (i.e. extension or intensity of the colour) could be used as a visual cue to assess individual quality. The key functional question is as follows: what determines the variability in colour expression among individuals? In other words, what factors limit the deposition of carotenoids in beaks, skin, scales or feathers to maximize their colour? The answer to these questions demands a deep understanding of carotenoid physiology and metabolism (McGraw 2006a; Pérez-Rodríguez 2009; Pérez-Rodríguez et al. 2016). Avian species, like the above-mentioned zebra finch, the European greenfinch (*Carduelis chloris*) or the house finch (*Haemorhous mexicanus*), have been widely used to address these questions (McGraw 2006a). Although the red-legged partridge is a newcomer to that list of avian study models, it has provided really useful insights into the factors regulating carotenoid metabolism and carotenoid-based colourations.

Carotenoids in Teguments and Blood: Sources of Variation

The red colour of the beak, legs and eye rings of the red-legged partridge results, respectively, from the accumulation of red carotenoids in the ramphoteca and epidermis (Pérez-Rodríguez and Viñuela 2008; García-de Blas et al. 2011, 2013, 2014). Several red carotenoids (ketocarotenoids) can be found in these integuments. The most abundant is astaxanthin followed by papilioerythrinone, lutein and canthaxanthin (García-de Blas et al. 2013, 2014). Both astaxanthin and papilioerythrinone are mostly present (>80%) in esterified forms (mono- and diesters) (García-de Blas et al. 2013). By contrast, there are only two carotenoids in the plasma and internal tissues (liver, fat) of the red-legged partridge: lutein and zeaxanthin (García-de Blas et al. 2015, 2016). These two carotenoids are the most abundant in the diet of partridges and other granivorous and herbivorous birds (McGraw 2006a).

These descriptive data provided very relevant basic information about the carotenoid metabolism in the red-legged partridge. For instance, the fact that none of the main ornamental red carotenoids appear in the bloodstream or in internal organs implies that partridges metabolize dietary (yellow) precursor carotenoids into ornamental (red) carotenoids. Specifically, the most likely metabolic pathway for astaxanthin and papilioerythrinone production is the transformation of zeaxanthin and lutein, respectively (García-de Blas et al. 2014) (Fig. 2). More importantly, the lack of these two red ornamental carotenoids in blood or the liver indicates that the biotransformation of ingested dietary precursors into ornamental carotenoids takes place directly at the red integuments (García-de Blas et al. 2015). Interestingly, supplementation experiments have shown that increasing astaxanthin or canthaxanthin levels in the diet of partridges did not enhance, but reduced ornamental trait redness (García-de Blas et al. 2016; Alonso-Álvarez et al. 2018). Indeed, whereas canthaxanthin was absorbed and deposited in the red teguments, internal organs and egg yolks of partridges, dietary astaxanthin was not. The negative effect of dietary intake of these two ketocarotenoids on trait redness is probably due to an interference with the absorption of lutein and zeaxanthin and probably also vitamin E. This is interesting from an ecological perspective, as lutein and zeaxanthin (but not astaxanthin and canthaxanthin) form a significant part of the natural diet of this species in the wild. Thus, the metabolic pathway towards an intense red colouration in this species does not admit shortcuts: only increasing the intake of the dietary

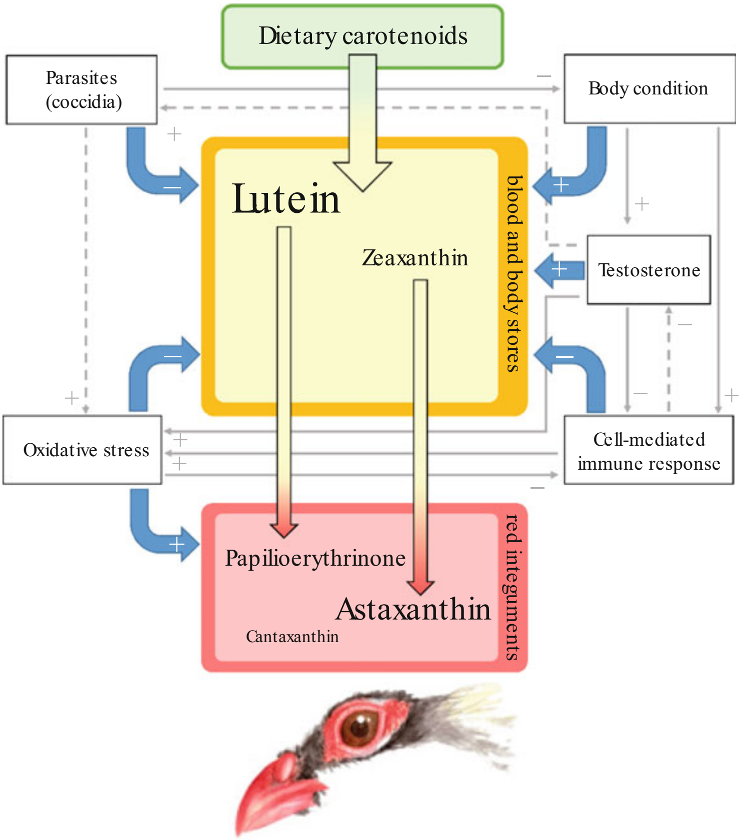

Fig. 2 Physiological pathway of carotenoid-based colouration in the red-legged partridge and factors that affect the different phases. The size of the name of each carotenoid type represents its relative abundance in each compartment. Solid grey arrows indicate interactions between factors that have been proved in this species, either by experimental or correlational evidence; dashed grey lines represent interactions found in similar species but still untested in the red-legged partridge. See the main text for details. Illustration credit: Lorenzo Pérez-Rodríguez

precursors of the main ketocarotenoids pigmenting their legs, beaks and eye rings can allow partridges to maximize their colouration. This is consistent with the "carotenoid limitation" —or "foraging ability"— hypothesis for the evolution of carotenoid-based traits as signals of individual quality (Endler 1983; Hill 1990; McGraw 2006a). According to this hypothesis, carotenoids are a limited resource for most animals, and better foragers would be able to obtain a higher amount of carotenoid precursors to maximize the colour intensity of their ornaments. In this way, ornament colouration would directly reflect foraging efficiency and nutritional status, serving as an honest indicator of these relevant fitness-related traits. Consistently with this idea, at least in captive conditions, the overall nutritional condition of red-legged partridges is positively related to the red pigmentation of their beaks and eye rings and negatively to the physiological stress experienced by the individual (Pérez-Rodríguez and Viñuela 2008; Mougeot et al. 2009). Confirmatory evidence that the same holds true under natural scenarios —where the carotenoid content of diet is probably higher— is still pending.

The detailed knowledge on biochemical pathways leading to the red traits also allows for a further refinement of the carotenoid limitation hypothesis (García-de Blas et al. 2014; Pérez-Rodríguez et al. 2016). Thus, astaxanthin, the most abundant carotenoid in the red teguments of the red-legged partridge as well as the main responsible of the redness of the trait (García-de Blas et al. 2013, 2014), results from the transformation of zeaxanthin, the least abundant carotenoid in the partridge diet (García-de Blas et al. 2015, 2016). This might impose an extra handicap to the ornament pigmentation, reinforcing the honesty of red ornaments. Also, the metabolic pathways implicated in the synthesis of red carotenoids would entail certain physiological costs (see below), contributing to make the reddest colouration unaffordable for low-quality birds. Importantly, astaxanthin is the main carotenoid in red colourations in many animal species (not just in birds), which would make the conclusions obtained for the red-legged partridge applicable to many other groups (e.g. Wedekind et al. 1998; Pérez-Rodríguez et al. 2016).

Wild and farm-reared partridges often differ in the intensity of their red colouration, as largely known by hunters and gamekeepers and as supported by scientific evidence (García-de Blas et al. 2013). These differences do not result from the presence of different carotenoids in their internal organs and ornaments, but mostly from differences in the relative and absolute concentrations of the two ketocarotenoids mentioned above (García-de Blas et al. 2013, 2015). Unfortunately, a detailed description of the carotenoid composition of the red-legged partridge's diet in the wild is still pending. Therefore, we do not know if the redder colouration of wild birds as compared to captive ones is due to the higher overall carotenoid content of their diet, to a relatively higher proportion of zeaxanthin over lutein, to a higher absorption capacity and carotenoid metabolism efficiency of wild birds, or to a summed effect of all these factors. In any event, absolute and relative ketocarotenoid concentration in ornamental red traits may not be the only factor contributing to colour differences between wild and captive partridges. For instance, it has been observed that ornamental carotenoids are present in higher levels of esterification in wild than in farm-reared birds, which could also contribute to enhance the colour stability and intensity in the former (García-de Blas et al. 2013).

Although dietary carotenoid intake is a major determinant of carotenoid-based colouration, variability in colour expression remains when carotenoid intake is constant. For instance, higher levels of carotenoids are found in the blood of males as compared to females fed the same diet (Negro et al. 2001; Pérez-Rodríguez 2008; Pérez-Rodríguez and Viñuela 2008), which results in subtle but detectable differences in colouration between sexes (Villafuerte and Negro 1998; Pérez-Rodríguez 2008; Pérez-Rodríguez and Viñuela 2008). Also, circulating carotenoids and carotenoid-based colouration vary seasonally, peaking during the mating season and decreasing afterwards (Pérez-Rodríguez 2008; Alonso-Álvarez et al. 2008), thus reflecting the dynamic nature of these traits (Pérez-Rodríguez 2008). Such decrease in plasma carotenoid levels is particularly marked in females, who must allocate a great amount of these pigments to the egg yolk production (Bortolotti et al. 2003; Pérez-Rodríguez 2008). However, similar—but less marked—temporal dynamics are observed in males, reflecting seasonal changes in the allocation and/or requirements of these pigments. Also, some studies have reported age-related variations, with lower circulating carotenoids and duller carotenoid-based colouration in older birds, at least in captivity (Alonso-Álvarez et al. 2009, 2010; García-de Blas et al. 2013).

The high degree of inter-individual variability in carotenoid-based colouration not attributable to the diet is not restricted to the red-legged partridge, but has been widely reported in many species of birds and other animals. This was identified by researchers as a clear evidence that additional factors (other than carotenoid access) mediate the expression of carotenoid-based signals, which would ultimately imply that these traits would signal *something else* than foraging ability and nutritional status (Lozano 1994; von Schantz et al. 1999). For that reason and considering that carotenoids have many biochemical roles apart from serving as integumentary pigments, researchers have formulated a series of new hypotheses trying to explain the function and evolution of these traits as signals of quality (Lozano 1994; von Schantz et al. 1999; McGraw 2006a; Pérez-Rodríguez 2009; Hill and Johnson 2012) (Fig. 1). In this context, the red-legged partridge has served as a very useful model for the study of honest colour signals.

Physiological Regulation of Carotenoid-Based Signals

Once carotenoids have been acquired through diet, carotenoid-based signal expression can be constrained in at least three main ways. First, carotenoid absorption, mobilization or transformation would require the activation of certain mechanisms (e.g. endocrine, metabolic) that would, in turn, entail certain costs for the individual, decreasing its fitness. Second, the use of carotenoids for integument pigmentation would consume a relevant portion of the physiological stock of these molecules, potentially impairing the functionality of other processes that also require the participation of carotenoids. This situation would represent a trade-off in the use of carotenoids for colour signalling versus self-maintenance, and only high-quality individuals would satisfy adequately both branches of the dilemma. The third pathway is related to external factors or internal processes that would cause, as a side effect, a reduction in carotenoid bioavailability or carotenoid metabolism efficiency, ultimately resulting in a reduction of carotenoid deposition in the ornament (Pérez-Rodríguez 2009; Hill 2011). These hypotheses are not mutually exclusive; in fact, they share common mechanisms and processes (e.g. action of the endocrine system, immune function, redox balance), with dynamic interactions that are difficult to disentangle (Pérez-Rodríguez 2009). However, it is necessary to assess the relative importance of each physiological mechanism separately because the theory predicts that the information provided by a given signal must be unavoidably related to its production costs and constraints. Studying the physiological mechanisms implicated in carotenoid bioavailability and use is therefore essential to fully understand the evolution of carotenoid-based signals. The advent of the red-legged partridge as a valuable study model for carotenoid-based traits coincided with the emergence of these physiological hypotheses. For this reason, much of the research done on this species addressed these topics, resulting in highly relevant contributions to this field of research (see review in Pérez-Rodríguez et al. 2013a).

A pivotal concept in all these physiological mechanisms linked to carotenoid colouration is oxidative stress. Oxidative stress results from the imbalance between production of reactive oxygen species and the antioxidant defences of the organism in favour of the former (Halliwell and Gutteridge 2007). The main source of reactive oxygen species is the aerobic metabolism, although other sources like immune response or pollutants can also contribute significantly. Importantly, oxidative stress results in relevant damages to biomolecules such as DNA, lipids and proteins, impairing their functionality. Oxidative stress has become a popular concept for the general public in the last years due to its involvement in ageing and several degenerative diseases (Ahmad 1995; Barnham et al. 2004; Halliwell and Gutteridge 2007). But beyond its biomedical interest, oxidative stress has drawn the attention of evolutionary biologists due to its role in many biological processes, which makes it a good candidate for mediating different life-history trade-offs (von Schantz et al. 1999; Costantini 2008, 2014; Monaghan et al. 2009). This is particularly remarkable in the case of carotenoid-based signals, as carotenoids can be involved in redox status in several ways, either acting as antioxidants or just because they are particularly susceptible to oxidation by reactive oxygen species (Lozano 1994; von Schantz et al. 1999; Hartley and Kennedy 2004; Pérez-Rodríguez 2009; Simons et al. 2012).

Carotenoid-Based Traits as Indicators of Oxidative Stress and Immune Function

Studies addressing the links between carotenoid-based colouration and health-related variables (i.e. oxidative stress, immunocompetence or parasite levels) in the red-legged partridge have focused on the redness of the beak and eye rings. Given that sexual signals are expected to be particularly sensitive to diseases and infections (Hamilton and Zuk 1982; Balenger and Zuk 2014), special attention has been paid to the relationship between the redness of these traits and certain parasites, like coccidia. Coccidia are parasitic protozoa that infect the caeca and small intestine of their hosts. Coccidiosis is a common infection in poultry but also affects wild avian populations. High levels of coccidia cause a reduction of circulating carotenoids in the red-legged partridge, thus decreasing the carotenoid pigmentation of the eye rings and beak (Mougeot et al. 2009). Similar negative impacts of coccidia on carotenoid-based colouration have also been reported in other bird species (e.g. Brawner et al. 2000; McGraw and Hill 2000; Horak et al. 2004; Martinez-Padilla et al. 2007). The most likely explanation for this negative effect is that coccidia infection damages the intestinal epithelium, thereby reducing carotenoid absorption (Allen 1987), with a subsequent fading in colouration. However, the negative impact of parasites on carotenoid-based colouration may also result from another indirect pathway. Infections elicit an immune response from the host, which may itself consume carotenoids. In fact, experiments in the red-legged partridge have revealed that cell-mediated immune responses reduce circulating carotenoids (Pérez-Rodríguez et al. 2008), which would potentially decrease redness of the teguments if the immune challenge is maintained in time. This is attributed to the direct implication of available carotenoids on immune responses, as these molecules exhibit immunostimulant properties (Simons et al. 2012). Again, observational evidence in the red-legged partridge supports this hypothesis, as cellular immunity is positively related to circulating carotenoid levels and redness in this species (Blas et al. 2006; Pérez-Rodríguez et al. 2008; Mougeot et al. 2009). Altogether, these results indicate that carotenoid-based ornamentation in the red-legged partridge—at least eye rings and beak colouration—would be informative of the sanitary state of the individual and the ability to face future immune challenges (Pérez-Rodríguez et al. 2008, 2010; Mougeot et al. 2009) (Fig. 1). Indeed, a long-term study in captivity showed that the intensity of the red colouration predicted both longevity and lifetime fecundity in male partridges (Cantarero et al. 2019), supporting the idea that red ornaments reflect the overall health and viability of individuals.

Much of recent research on carotenoid physiology produced in the last years has focused on the potential antioxidant role of carotenoids (Pérez-Rodríguez 2009). In fact, the mentioned link between carotenoids and immune response in the partridge —in consistence with results from other species— could also be attributed to a mediating role of oxidative stress. Immune responses entail a release of reactive oxygen species, and carotenoids would be consumed as antioxidants during the challenge (Halliwell and Gutteridge 2007; Costantini and Moller 2009). Evidence from the red-legged partridge offers some support for such an antioxidant role. Although circulating carotenoids are mostly unrelated to oxidative damage biomarkers in observational studies (Alonso-Álvarez et al. 2008, 2010; Pérez-Rodríguez et al. 2008), experimental setups revealed a different picture. When partridges are fed with high vs. low doses of carotenoids, high carotenoid intake seems to confer some protection against an oxidative challenge elicited by a regular intake of diquat—a free radical generator—at least in females (García-de Blas et al. 2016). It seems therefore that carotenoids can offer some antioxidant protection in this species. But, does the reverse hold? Does oxidative stress impair carotenoid availability, subsequently fading colouration? The empirical evidence in this sense draws a more complex scenario (Fig. 2). Experiments exposing red-legged partridges to oxidative challenges via diquat administration have reported contrasted results. In juveniles, oxidative challenge leads to a decrease in circulating carotenoids and carotenoid-based colouration (Alonso-Álvarez and Galván 2011). However, a similar oxidative challenge applied to adults produced no effects on carotenoid levels in blood or internal tissues, but increased ketocarotenoid concentration in ornamental traits, enhancing their redness (García-de Blas et al. 2016). These contrasted results would arise from a higher susceptibility to oxidative stress of juveniles, whose antioxidant system would still be relatively immature. But more interestingly, the unexpected positive effect of oxidative challenge on redness found in the second study led to a novel hypothesis (García-de Blas et al. 2014, 2015, 2016). Given that the transformation of lutein and zeaxanthin into papilioerythrinone and astaxanthin requires some oxidation steps, researchers proposed that a certain level of oxidative stress favours the in situ biotransformation of dietary into ornamental carotenoids (García-de Blas et al. 2016). This may also require the activity of the oxygenase CYP2J19, an enzyme that is part of the P450 cytochrome, which is involved in many detoxification reactions (Lopes et al. 2016; Mundy et al. 2016). This would support the hypothesis that carotenoid-based colouration could be a sign of the capacity of a given individual to manage oxidative stress, as both carotenoid metabolism and detoxification ability would share common pathways (Hill and Johnson 2012; Johnson and Hill 2013). Interestingly, it has been shown that the eye ring redness predicts the resistance to future immune challenges in the red-legged partridge, as redder individuals suffer less oxidative damage when mounting an immune response (Pérez-Rodríguez et al. 2010). The results obtained in red-legged partridges could be extrapolated to other species also displaying red carotenoid-based ornaments with the similar biochemical profile (e.g. Pérez-Rodríguez et al. 2016).

Testosterone-Mediated Control of Carotenoid-Based Traits

The steroid hormone testosterone plays a key role in regulating the expression of sexual characters, enhancing the development of typical male traits and behaviours that are often displayed as signals of quality (Adkins-Regan 2005). However, such signal-enhancing effect may entail costs, as high levels of testosterone would lead to immunosuppression (Folstad and Karter 1992). These contrasted effects of the hormone are the two pillars underpinning the immunocompetence handicap hypothesis, which suggests that only high-quality individuals are able to afford the immunosuppressive effects of testosterone while showing the most elaborated sexual displays (Folstad and Karter 1992). There is a large body of literature testing this hypothesis, which is still a subject of debate (Roberts et al. 2004). Furthermore, some modifications of the original hypothesis have been put forward, like the so-called oxidation handicap hypothesis, which proposes that oxidative stress, rather than —or in addition to— immunosuppression, would be a plausible cost of testosterone level ultimately ensuring the reliability of sexual signals (Alonso-Álvarez et al. 2007).

An experiment in captivity has provided compelling evidence of the viability costs of testosterone for male partridges (Alonso-Alvarez et al. 2020). In that study, 1-year-old males were treated with testosterone-filled subcutaneous implants, whereas others (controls) were treated with empty implants. The experiment was repeated every following breeding season, for 6 years. Males treated with testosterone lived shorter than controls, since they were more prone to die from a natural bacterial infection. Whether this effect is due to a direct immunosuppressive effect of the hormone or to an indirect effect of oxidative stress is unknown, however.

Although apparently unrelated, testosterone and carotenoids share many common features: both are usually higher in males as compared to females and in adults as compared to juveniles, and their levels are usually higher during the mating season. Also, as mentioned, both compounds are linked to social signalling and to immunocompetence. Despite such strong similarities, it was not until 2006 when a general connection between both compounds was established and experimentally demonstrated, precisely, using the red-legged partridge as study model (Blas et al. 2006). Working with captive partridges, researchers found that an experimental increase in testosterone levels was mirrored by an elevation of circulating carotenoids (Fig. 2). Interestingly, such an increase did not result from a mobilization of carotenoids from body stores but, apparently, from an increase in absorption capacity. Other studies have proposed that such increase in circulating carotenoids would also be favoured by a testosterone-mediated enhancement of transport molecules in the bloodstream, like cholesterol (McGraw et al. 2005). Irrespective of the proximate mechanism, carotenoid surplus may enhance the ability of individuals to develop an effective immune response under high testosterone levels. Such a link between carotenoids and testosterone underpins a new perspective for the honesty of carotenoid-based signalling, as high-quality individuals would need to allocate fewer proportions of these carotenoids to compensate for the immunosuppressive actions of testosterone, providing grounds to advertise their quality by devoting the carotenoid surplus to ornaments (Blas et al. 2006). Since the formulation of this hypothesis in the red-legged partridge, the regulation of carotenoid physiology by testosterone has received further support in other species (e.g. McGraw and Ardia 2007; Mougeot et al. 2007; Casagrande et al. 2011; Peters et al. 2012).

But we can still give another turn to the screw to the story. Testosterone upregulates metabolic rates, which may increase the production of reactive oxygen species and, ultimately, lead to oxidative stress (von Schantz et al. 1999; Alonso-Álvarez et al. 2007; Hill 2014). This, added to mentioned connections between testosterone, carotenoids and immunity, seems to support the central role of oxidative stress in mediating honesty of carotenoid-based colouration. Research conducted with the red-legged partridge has paid particular attention to the interplay among these factors. Experimental evidence in this species supports the existence of oxidative cost associated with high testosterone levels in males during mating (Alonso-Álvarez et al. 2008). Carotenoid bioavailability and colour expression are influenced by such costs, but, interestingly, the impact of these costs largely depends on the quality of the individual (as expected, in fact, from a reliable signalling system; Zahavi 1975). Thus, 2-year-old partridges, which are likely at the apogee of their vigour, responded to an experimental increase in testosterone levels by elevating their circulating carotenoids and enhancing the redness of their eye rings (Alonso-Álvarez et al. 2009). By contrast, old males—presumably at the decline of their somatic integrity—were negatively affected by increased testosterone levels, showing decreased circulating carotenoids and ornament redness when exposed to high levels of the hormone. Furthermore, whereas middle-aged males showed no adverse effects of the treatment, old males treated with exogenous testosterone elicited weaker immune responses (Alonso-Álvarez et al. 2009). Circulating carotenoids and the higher levels of oxidative stress experienced at old ages apparently mediated these age-dependent effects of testosterone on red ornamentation. Senescence is characterized by a deterioration of somatic condition. Thanks to these age-dependent effects of testosterone on oxidative stress and immunocompetence, a testosterone-mediated mechanism of colour expression would maintain the honesty of the signal, which would reliably mirror the current state of the individual.

In summary, current evidence suggests that the honesty of carotenoid-based traits in the red-legged partridge depends on several mechanisms (resource allocation trade-offs, hormonal mediators that impose physiological handicaps, shared pathways, side effects of the functionality of vital pathways, i.e. Hill 2011) whose relative importance may vary across life stages and environmental contexts.

Melanin-Based Traits of the Red-Legged Partridge Plumage

Although carotenoids have attracted a great interest of behavioural ecologists for decades, they are not the main pigment in animals. This pre-eminent position is occupied by melanins, which are responsible for many of the black, brown, grey and rufous patterns observed in animals (as well as plants and fungi). This wide array of colours is the result of variations in the relative and absolute concentrations of the two main types of melanins: eumelanin (black) and pheomelanin (reddish-brown) (McGraw 2006b). However, unlike carotenoid-based colourations —which are supposed to evolve mostly as signalling traits—, melanin-based colourations often entail multiple functions unrelated to communication, including thermoregulation, camouflage or enhancement of the mechanic endurance of teguments. Nonetheless, melanin-based traits also play a significant role as signals (McGraw 2006b), but the mechanisms regulating their reliability are still poorly known (Jawor and Breitwisch 2003; McGraw 2006b) as compared to carotenoids. One key difference between carotenoids and melanins is that the later are synthesized by animals. This has led to the largely sustained assumption that melanins are relatively inexpensive to produce (Jawor and Breitwisch 2003; McGraw 2006b), apparently discarding the condition-dependent expression of melanin-based traits. However, several non-mutually exclusive hypothetical mechanisms would explain a quality-dependent expression of melanin-based traits: the social control of traits used as badges of status (Senar 2006), the pleiotropic effects of the physiological pathways involved in melanin synthesis (Ducrest et al. 2008), the potential oxidative costs of eumelanin synthesis (Galván and Alonso-Álvarez 2008; Galván and Solano 2009) or the condition-dependent expression of melanin-based patterns (Bortolotti et al. 2006; Griffith et al. 2006; Pérez-Rodríguez et al. 2013b; Pérez-Rodríguez et al. 2017).

As said at the beginning of this chapter, the plumage of the red-legged partridge is composed of a combination of drab tones and conspicuous badges that likely reflect an evolutionary trade-off between camouflage and signalling. Chemical analyses of the melanin composition of the different plumage patches are still pending (Toral et al. 2008), but their perceived colour can be used as a raw approximation to its composition (Galván and Wakamatsu 2016). Brownish olive hues of the back plumage likely result from a balanced content of eu- and pheomelanin in the feathers. By contrast with the mimetic back, the red-legged partridge shows an orange belly (based on pheomelanin), a black bib (based on eumelanin) and a conspicuous lateral barred pattern composed of an alternation of whitish, black and brown bars—unpigmented, eumelanin- and pheomelanin-based, respectively—within each feather. The study of the lateral barred plumage and, specially, the black bib has revealed significant links with individual quality, which suggests a potential role as signals of quality (e.g. Bortolotti et al. 2006; Pérez-Rodríguez et al. 2013b) (Fig. 1). Interestingly, both plumage traits are conspicuously displayed by individuals during mating and agonistic interactions (Cramp and Simmons 1980), which is consistent with their putative role in social signalling.

Melanin-Based Plumage Patches: Signals of Oxidative Stress and Condition?

There is evidence that some melanin-based plumage ornaments of the red-legged partridges are connected to nutritional status. For instance, the size of the black bar of the flank feathers is related to the body condition of the individual (Bortolotti et al. 2006), suggesting a potential role of this trait as a signal of nutritional status (Fig. 1). However, the mechanistic link between condition and melanin production remains elusive (Griffith et al. 2006; McGraw 2006b). A constraint based on the relative scarcity of some essential micronutrients required for eumelanin synthesis or the existence of certain allocation trade-offs has been proposed, but sound and general empirical evidence of these mechanisms is still missing (McGraw 2008).

Although much less developed than in the case of carotenoids, a physiological link between oxidative stress and melanin-based colour expression has also been proposed (Galván and Alonso-Álvarez 2008; Galván and Solano 2009). According to this framework hypothesis, the melanogenic pathway involves a relatively complex series of interactions among different compounds, where glutathione plays a central role (Galván and Alonso-Álvarez 2008; Galván and Solano 2009). Glutathione is a peptidic molecule that inhibits the eumelanin pathway (Galván and Solano 2009). Thus, the levels of this key molecule seem to regulate the synthesis (amount and type) of melanin within the melanocyte. But, importantly, glutathione also plays a central role in other processes. For instance, glutathione is the main endogenous nonenzymatic antioxidant in the cell, protecting its functional integrity against free radicals (Halliwell and Gutteridge 2007). This dual role of glutathione is the basis of the hypothesised physiological link between melanin pigmentation and oxidative stress: high oxidative stress would deplete glutathione levels, thus favouring eumelanin synthesis, whereas low oxidative stress levels will lead to high glutathione levels, inhibiting eumelanin synthesis. Thus, according to this hypothesis, eumelanic signals would be expressed at their highest intensity only when the individual is able to counteract the cost of low glutathione levels by activating alternative antioxidant mechanisms. In other words, eumelanin-based traits would evolve as honest signals of the functionality of the antioxidant system. The hypothetical mechanism assuring pheomelanic signalling is however less evident and dependent on resource allocation trade-offs as this melanin depends on cysteine availability in the diet (Galván and Solano 2009).

This mechanism thus sets the ground for a context-dependent reliability of eu- and pheomelanin colourations that still awaits experimental support (Galván and Solano 2009). So far, the red-legged partridge constitutes one of the best examples supporting the connection between oxidative stress and melanin-based pigmentation. In an experiment performed on partridge chicks of approximately 1 month of age, researchers found that increasing individual oxidative stress by administration of an exogenous source of free radicals (diquat) reduced glutathione levels and led to larger eumelanin plumage ornaments, i.e. increased size of the black bib and average area of the black band of flank feathers. By contrast, the same treatment reduced the area of the brown-reddish pheomelanin-based band of the flank feathers (Galván and Alonso-Álvarez 2009). Thus, these results support the proposed link between oxidative stress and plumage pigmentation, with glutathione levels as a key mediator of the process. Given the close links between oxidative stress and individual performance (see above), this opens a window to consider the size or intensity of melanin-based traits of the red-legged partridge as an indicator of individual oxidative status. Note, however, that the connection is not simple and intuitive, as situations of high oxidative stress might *facilitate* the production of black (eumelanin-based) traits, making them relatively affordable for all individuals, i.e. less reliable signals. This contrasts with the above-mentioned positive links between eumelanin-based pigmentation and condition found in this species. Therefore, the potential context-dependent meaning of these traits deserves further research.

A Matter of Pattern Expression?

The traditional approach to the evolution of colour signals assumes that pigment acquisition or production and its allocation to external teguments are the main constraints of signal expression. This implicitly assumes that colour intensity and the size of the patches are the key features conveying information on individual quality. However, this traditional paradigm has been recently challenged, as evidence from diverse taxa suggests that the actual pattern (i.e. shape of a patch, uniformity of the borders, regularity or complexity of the design) would equally behave as a reliable signal of individual quality in contexts of social communication (Pérez-Rodríguez et al. 2017). Some empirical evidence on this emerging research line has resulted from research on the red-legged partridge. As said before, the lateral barred pattern of this species results from the perfect alignment of flank feathers. Although there can be notable variations in the width of the bands, a detailed study of this trait reported that the number of bands remains relatively invariable among individuals (8–9 in more than 90% of individuals of either sex) (Bortolotti et al. 2006). Interestingly, despite covering a relatively large body area (ca. 84 cm^2), the feathers creating the pattern (roughly 45–50) emerge from a small piece of the skin about 1x4 cm on the upper breast. This implies that some of these ornamental feathers —particularly those covering the rear section of the patch— are notably long, exceeding 9 cm. The alignment of the bands and their position during courtship and agonistic behaviours are regulated by muscular contraction acting on feather follicles, creating a dazzling visual display. The offset of such display architecture is its susceptibility to alterations due to feather loss. Flank feathers —particularly the longest ones— are in fact loosely attached to the bird's body and therefore can be easily lost during agonistic encounters or when eluding a predator grasp, through a kind of autotomy strategy (Moller et al. 2006). The presence of such composite barred pattern might have evolved as an amplifier of somatic integrity, whose alterations can be used as cues for individual quality assessment (Pérez-Rodríguez et al. 2017). Interestingly, when any of these flank feathers is lost, replacement feathers do not perfectly fill these gaps. As a result, the pattern reveals long-lasting traces of recent traumatic events that would otherwise remain unnoticed by conspecifics and cannot be repaired until the next natural moult (Bortolotti et al. 2006).

The spatial configuration of the black bib of the red-legged partridge also conveys information on individual quality (Pérez-Rodríguez et al. 2013b). In a study combining observational and experimental data from captive partridges, researchers found that birds displaying bibs with higher fractal dimension —characterized by a smooth transition between the uniform black throat patch and the lower spotted region of the trait— showed better body condition and stronger immune responses within the study population. Also, when individual body condition was experimentally impaired during moult, new bibs showed a lower fractal dimension than bibs previously displayed by the same birds, while control (ad libitum-fed) individuals moulted bibs with a similar fractal dimension (Pérez-Rodríguez et al. 2013b). These results in captivity have been complemented by evidence from wild birds during the mating season. Thus, mated birds of both sexes showed higher bib fractal dimension than unmated ones (Cantarero et al. 2018). Moreover, paired individuals showed assortative mating according to the fractal dimension of their bibs, which can be considered as an indirect evidence of the implication of this character in mate choice (Cantarero et al. 2018). These results indicate that, irrespective of the size of the bib (a proxy of the amount of melanin deposited in the trait), the pattern of this trait conveys information about individual quality and is actively used as a social signal in this species. It remains unknown, however, what is the mechanistic and functional link between individual quality (i.e. nutritional status, immunocompetence) and pattern expression. Creating colour patterns requires a tight control over multiple mechanisms that must be synchronized at very different spatial and temporal scales. One possibility is that factors affecting developmental stability or homeostasis during moult may alter this machinery, resulting in changes in the expression of the pattern. Complex colour patterns, like the bib of the partridge, may therefore act as indices of the individual capacity to buffer stressors that alter these vital processes, thus linking individual quality and trait expression (Pérez-Rodríguez et al. 2017).

Future Research Directions

Although a newcomer to the evolutionary ecology scene, the red-legged partridge has contributed to the advance of this field of research by serving as an ideal study model for several sub-disciplines. Most of these contributions have focused on the physiological regulation of carotenoid-based colouration, particularly on its links with oxidative stress and androgens. In this sense, the findings obtained in this species have challenged the long-standing paradigm of the resource allocation trade-off as the main physiological way to explain the honesty of carotenoid-based signals. More recently, research on melanin-based traits has contributed to improve our understanding of the factors affecting the expression of these colour traits in vertebrates. However, we are only beginning to grasp the potential of this species as a model for research on these and other fields within evolutionary ecology. Unfortunately, the fast advance in these topics contrasts with the limited attention paid to other key issues that deserve proper assessment in order to allow a sound scientific progress. For instance, in contrast with our understanding of the biochemical basis and the mechanisms regulating carotenoid physiology, empirical evidences supporting the actual use of these traits in social interactions (i.e. mate choice or intra-sexual competition) are limited and rather indirect (Casas et al. 2010; Alonso-Álvarez et al. 2012). Experimental tests specifically designed for addressing the signalling value of these traits are needed to fully assess the biological relevance of the above-mentioned physiological framework. The same applies to melanin-based plumage traits, as evidence supporting their role in social communication is only indirect and correlational (Cantarero et al. 2018). Obtaining such relevant information is feasible since mate choice trials and male-male dominance tests can be easily applied in partridges (e.g. Alonso et al. 2008; Prieto et al. 2018).

Although carotenoid- and eumelanin-based traits have attracted most attention from researchers interested in social signalling, other conspicuous traits of the red-legged partridge that may also play a significant role in communication have been overlooked. The orange belly plumage, for instance, shows a considerable degree of variability in chroma (*pers. obs.*), but whether this relates to age, sex or any aspect of individual quality remains unknown. The presence of such conspicuous pheomelanin-based trait that is jointly expressed with other eumelanin-based plumage patches mentioned above makes the red-legged partridge a perfect model species to evaluate contrasted responses for both types of melanins under different physiological and environmental contexts (Galván and Solano 2009). Apart from chromatic traits, this species presents another trait that is widespread among Galliformes: the spurs. Spurs are present in males as well as in some female partridges. However, to date, no study has explored the sources of variation of this trait. Behavioural tests are also required to assess their use as armaments or ornaments. Finally, the red-legged partridge exhibits a large set of ritualized behavioural displays and vocalizations (Pintos et al. 1985) whose signalling potential has been largely ignored and deserves a detailed research programme.

As shown in this chapter, different aspects of social signalling in the red-legged partridge have been thoroughly studied by evolutionary ecologists. However, it is necessary to broaden the scope of future studies to address additional aspects of the ecology and life history of the species. Understanding the factors underlying the observed variability in mating and breeding strategies of partridges (Green 1984; Casas et al. 2006a, 2009) is essential to fully understand the biology of the species. Also, such a broad picture will allow us to better interpret the relevance of the results reported in this chapter in an integrative ecological context. An extra effort to connect key life-history traits of the red-legged partridge with other relevant aspects, like its spatial ecology and sanitary issues (see Chaps. "Habitat Use and Selection: Is Habitat Management the Key to Restore Red-Legged Partridge Populations?" and "Health Monitoring and Disease Control in Red-Legged Partridges"), will ultimately allow a more efficient and integral design of management and conservation policies.

To date, most research on the evolutionary ecology of the red-legged partridge has been performed in captivity. The easy adaptation of this species to captive conditions and the existence of optimized protocols and adapted facilities derived from commercial breeding have facilitated long-term research programmes that addressed different aspects of its biology. However, the availability of these experimental setups and rearing facilities may also allow more ambitious studies that have been neglected to date. For instance, the establishment of selection lines for some of the physiological or phenotypic traits mentioned in this chapter would be of great interest for the study of the trade-offs associated with their expression, shedding light on the forces shaping their evolution. Similarly, the fact that several closely related *Alectoris* species can be easily maintained in captivity and cross-bred sets the ground for empirical setups to study the evolution of different traits within the genus. In any case, despite the great potential for captivity studies, the main challenge in the short term is to validate and calibrate the conclusions obtained in captivity with studies performed in the wild (e.g. García-de Blas et al. 2013). Comparison of partridge populations subjected to different management conditions —from unmanaged populations in relatively well-conserved natural habitats to highly managed populations in areas of intensive agriculture— may allow to test the potential context-dependent expression of the traits of interest.

Evolutionary ecology research using the red-legged partridge as a model species faces the challenge of dealing with a crossroad of diverse collectives with contrasted interests and perspectives. However, researchers must turn need into virtue and spotting the windows of opportunity that this situation offers, some of which have been highlighted above. Finding ways to interconnect the basic and applied research on this species also requires the involvement of other sectors of the society and governments. In this sense, the case of an emblematic game-bird species in the United Kingdom, the red grouse (*Lagopus lagopus scoticus*), is a good model to follow. In that case, scientists, hunters, gamekeepers and other stakeholders have collaborated for a long time in research programmes aimed at simultaneously resolving the basic evolutionary ecology questions as well as understanding key aspects of grouse biology of interest for population management, such as ecophysiology, sanitary status, behaviour, population dynamics or predator-prey interactions. This long-term intersectoral collaboration has been essential to implement a more efficient and science-guided management practices and conservation policies. The example of the red grouse is a proof that determined willingness for dialogue and cooperation, even though hard to reach, can generate highly beneficial synergies for all the involved sectors.

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