不同性别杂色山雀血浆活性氧和超氧化物歧化酶与繁殖成效的关系

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摘要：氧化应激是指活性氧等氧化剂的产生大于抗氧化防御系统清除能力时的不平衡状态，是个体应对内外环境刺激的适应性生理机制，是衡量个体身体状况的综合性生理指标。为探究氧化应激对鸟类繁殖的预测作用，本研究测量了育雏前期（雏鸟 6 ~ 8 日龄）杂色山雀（*Sittiparus varius*）亲鸟血浆氧化应激分子活性氧和超氧化物歧化酶含量，通过巢箱监测获得了杂色山雀的繁殖参数，采用偏最小二乘回归法分析杂色山雀氧化应激对其雏鸟出飞率和繁殖成功率的预测作用。结果显示，雌性杂色山雀亲鸟血浆活性氧浓度与雏鸟出飞率（$n = 13$，$P < 0.05$）、繁殖成功率（$n = 13$，$P < 0.01$）均呈显著负相关关系，即血浆活性氧水平越低的雌性杂色山雀，其繁殖巢雏鸟出飞率和繁殖成功率越高；雌性杂色山雀亲鸟血浆超氧化物歧化酶浓度与雏鸟出飞率（$n = 13$，$P > 0.05$）、繁殖成功率（$n = 13$，$P > 0.05$）无显著相关关系。本研究中这两项血浆氧化应激标记物与杂色山雀雄性亲鸟繁殖成效间均无显著的相关关系。该结果表明，杂色山雀雌性亲鸟活性氧水平对其繁殖成效具有显著的预测作用。

关键词：氧化应激；活性氧；超氧化物歧化酶；繁殖成效；杂色山雀

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The Relationships of Reproductive Success with Different Genders' Plasma Reactive Oxygen Species and Superoxide Dismutase in the Varied Tit (*Sittiparus varius*)

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Abstract: The difference in bird's body condition may lead to the variation of reproductive success. Recent works have suggested that biomarkers of oxidative stress might provide an additional tool to assess the health state of individuals. Oxidative stress is usually defined as the imbalance between pro-and anti-oxidative processes. Oxidative stress is the physiological adaptation mechanism of individuals to cope with stress, that

reflects the environmental conditions that birds experience. The oxidative status also a priori informative tool to evaluate the reproduction and survival of individuals. However, the conclusion about the role of oxidative stress on the reproductive of wild organisms is not consistent. In this study, to explore whether the plasma biomarkers of oxidative stress can predict the reproductive success of Varied Tit (*Sittiparus varius*), we took blood samples from the pairs of Varied Tit bred in the artificial nest boxes during their early brooding period (6‑8 days post-hatching). Then we used the ELISA kit to measure the levels of plasma oxidative stress (reactive oxygen species, ROS and superoxide dismutase, SOD concentration). We also got the reproductive parameters of Varied Tit by nest monitoring. Partial least squares regression (PLS) analysis was used to analyze the relationship between oxidative stress and reproductive success (fledging rate and breeding success). We found that there are no significant differences between males and females in plasma reactive oxygen species and plasma superoxide dismutase concentration during chick-rearing period (independent-samples *t*-test: reactive oxygen species, $t_{(20, 25)} = -0.887$, $P = 0.380$; superoxide dismutase, $t_{(20, 25)} = -1.325$, $P = 0.192$). We also found that the plasma reactive oxygen species concentration was significantly negatively correlated with the fledging rate (partial least squares regression: $B = -0.966$, $P = 0.013$, Fig. 1 a) and breeding success (partial least squares regression: $B = -0.976$, $P = 0.002$, Fig. 2 a) of female Varied Tits, and females with a lower reactive oxygen species concentration tended to have a higher fledgling rate and breeding success. The reproductive success of female Varied Tit was not related to the plasma superoxide dismutase concentration, and there was no statistical significance in any analysis of males. Our findings suggest that reactive oxygen species, as a biomarker of oxidative stress in plasma, could be a tool to assess the fitness components and predict the reproductive success of female Varied Tits.

Key words: Oxidative stress; Reactive oxygen species; Superoxide dismutase; Reproductive success; Varied Tit, *Sittiparus varius*

　　繁殖是鸟类生活史的关键环节，是鸟类生态学研究的重点领域。繁殖成效存在个体差异，这是物种进化的动力，也是鸟类繁殖生态学研究的重点之一。繁殖成效的差异可能与鸟类身体状况有关（Chastel et al. 1995，Milenkaya et al. 2015）。身体质量较高的个体更擅长获取和分配资源，因此具有更高的繁殖适合度。然而，目前对个体身体质量的评价多采用形态学参数，对个体生理指标的关注则相对较少。

　　氧化应激（oxidative stress）在预测个体适合度方面潜力巨大，是评价个体身体状况的综合性生理指标（Beaulieu et al. 2014，Hau et al. 2015）。氧化应激被定义为机体内活性氧（reactive oxygen species，ROS）等氧化剂的产生大于抗氧化防御系统清除能力时的不稳定状态（Sies 1985，Finkel 2000），受内源性代谢和

环境条件调控（Metcalfe et al. 2010），是个体面对各种环境压力和生活史挑战的生理适应性反应。如对歌带鹀（*Melospiza melodia*）（Travers et al. 2010）、塞岛苇莺（*Acrocephalus sechellensis*）（van de Crommenacker et al. 2011）、日本鹌鹑（*Coturnix japonica*）（Sahin et al. 2012）和乌鸫（*Turdus merula*）（Norte et al. 2018）等的研究表明，鸟类的氧化应激水平受到捕食压力、温度、栖息地质量及寄生虫感染等因素的影响。黑顶白颊林莺（*Dendroica striata*）和红眼莺雀（*Vireo olivaceus*）循环抗氧化能力与体现鸟类营养储备的脂肪评分正相关（Skrip et al. 2015）。此外，繁殖期鸟类较高的能量投入也可能引起氧化状态的改变，如产卵早的大山雀（*Parus major*）谷胱甘肽过氧化物酶活性更低（Norte et al. 2010）。因此，氧化应激状态是个

体代谢、压力水平、营养状况、免疫功能及身体素质的综合反映。

氧化应激作为个体适合度的衡量指标，在预测个体繁殖成效方面的作用也备受关注。但目前的研究中，关于氧化应激能否预测鸟类繁殖的结论并不一致。Cohen 等（2009）发现，孵卵期总抗氧化能力越强的白腰叉尾海燕（*Oceanodroma leucorhoa*），孵化成功率越高，而稀树草鹀（*Passerculus sandwichensis*）亲鸟孵卵期的总抗氧化能力与其繁殖率无关。漂泊信天翁（*Diomedea exulans*）繁殖前期氧化损伤与孵化率和出飞率无关（Costantini et al. 2015a）。目前，多数研究对氧化应激的评价是基于氧化或抗氧化单方面的，而事实上，两者的变化是相互影响的。如机体内重要的抗氧化酶——超氧化物歧化酶（superoxide dismutase，SOD）可加速超氧负离子发生歧化作用，生成过氧化氢并最终被分解为水和氧气，其含量可能随着机体活性氧产生的增加而被消耗减少，也可能随着机体活性氧产生减少而合成减少。因此，在评价氧化应激时，必须要同时关注氧化及抗氧化两个方面（Costantini et al. 2009，Selman 2012）。

杂色山雀（*Sittiparus varius*）是小型雀形目鸟类，社会性单配制，由雌鸟筑巢、雌鸟孵卵，双亲共同育雏，平均窝卵数 7 枚，平均孵卵时长 14 d，平均出飞日龄 18 日龄。目前，关于杂色山雀繁殖生态学研究主要集中于亲鸟身体质量对个体配偶选择、繁殖投入、繁殖成效及婚外配的影响。如景春雷等（2019）研究发现，雌性杂色山雀亲鸟的喙宽与其递食率呈极显著负相关。在衡量亲鸟身体质量方面，研究多以体重、跗跖长、翅长、尾长、喙长、喙宽等体征参数为衡量指标。本研究以个体氧化应激水平作为评价鸟类身体状况的指标，分析杂色山雀氧化应激水平与繁殖成效的关系。通过测定育雏前期杂色山雀亲鸟血浆活性氧和超氧化物歧化酶水平，分析氧化应激状态与雌雄杂色山雀子代出飞率及繁殖成功率的关系，以探究氧化应激作为衡量身体状况的指标对其繁殖成效的预测效果。

1　研究方法

1.1　繁殖数据收集

本研究通过在辽宁省仙人洞国家级自然保护区（122°53′24″ ~ 123°03′30″ E，39°54′00″ ~ 40°03′00″ N）内悬挂大量人工巢箱（长 × 宽 × 高为 16 cm × 14 cm × 34 cm），招引杂色山雀繁殖。在 2019 和 2020 年，每年均从 3 月下旬开始，每周巡箱 1 或 2 次，发现巢箱被杂色山雀利用后，每 1 ~ 3 d 巡查一次，记录其繁殖参数，包括窝卵数、出雏数和出飞数，并计算雏鸟出飞率（即出飞数与出雏数之比）和繁殖成功率（即出飞数与窝卵数之比）。

杂色山雀只有雌鸟孵卵，因此可根据孵卵斑区分亲鸟性别并环志（Bailey 1952）。在雏鸟6 ~ 8 日龄时，通过巢箱捕鸟器捕捉杂色山雀双亲（Zhang et al. 2019），每只亲鸟个体通过翅下肱静脉取血 50 µl，将血液置于经过肝素钠抗凝处理的 EP 管内，保存于液氮中。

本研究共收集 45 只杂色山雀血液样本，包括 20 只雌性血液样本（其中 13 只个体繁殖成功）和 25 只雄性血液样本（其中 17 只个体繁殖成功）。

1.2　氧化应激参数测定

采用 Avian ROS ELISA 试剂盒（KT5118-A，江苏科特生物科技有限公司）和 Avian SOD ELISA 试剂盒（KT5589-A，江苏科特生物科技有限公司）分别测定杂色山雀亲鸟血浆中活性氧和超氧化物歧化酶浓度。

1.3　统计分析

采用 R 4.0.3 软件对数据进行分析，所有分析显著性水平设置为 0.05。采用独立样本 *t* 检验（independent-samples *t* -test）分析种群内雌雄杂色山雀间活性氧和超氧化物歧化酶浓度的差异性。相关性分析显示，活性氧与超氧化物歧化酶具有强相关性，为避免共线性影响，采用偏最小二乘法（partial least squares regression，

PLS）分别分析雌雄杂色山雀活性氧和超氧化物歧化酶水平与繁殖成效的关系。

2 结果

2.1 活性氧与超氧化物歧化酶浓度

采用独立样本 t 检验分析结果显示，育雏前期雌性与雄性杂色山雀间血浆氧化应激标记物活性氧（$t_{(20, 25)} = -0.887$，$P = 0.380$）及超氧化物歧化酶（$t_{(20, 25)} = -1.325$，$P = 0.192$）浓度均无显著性差异。

2.2 杂色山雀亲本氧化应激与繁殖成效的关系

采用偏最小二乘法分析，雌性杂色山雀血浆活性氧水平与其子代出飞率呈显著负相关（$B = -0.966$，$t = -2.908$，$n = 13$，$P = 0.013$，图 1a），即血浆活性氧水平越低的个体本巢雏鸟出飞率越高。雌性杂色山雀超氧化物歧化酶水平与子代出飞率无显著相关性（$B = 0.678$，$t = 1.719$，$n = 13$，$P = 0.111$，图 1b）。雄性杂色山雀活性氧、超氧化物歧化酶水平与出飞率均无显著相关性（活性氧：$B = -0.215$，$t = -0.378$，$n = 17$，$P = 0.710$，图 1 a；超氧化物歧化酶：$B = 0.283$，$t = 0.425$，$n = 17$，$P = 0.676$，图 1b）。

雌性杂色山雀血浆活性氧水平与繁殖成功率呈极显著性负相关（$B = -0.976$，$t = -3.796$，$n = 13$，$P = 0.002$，图 2a），即血浆活性氧越低的雌性杂色山雀繁殖成功率越高。超氧化物歧
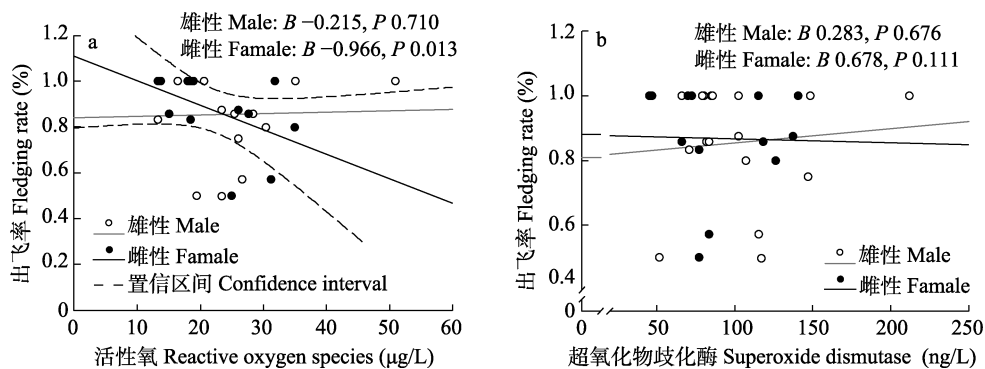

图 1 杂色山雀活性氧、超氧化物歧化酶与出飞率的关系

Fig. 1 The relationship between reactive oxygen species and fledging rate, and between superoxide dismutase and fledging rate of Varied Tit
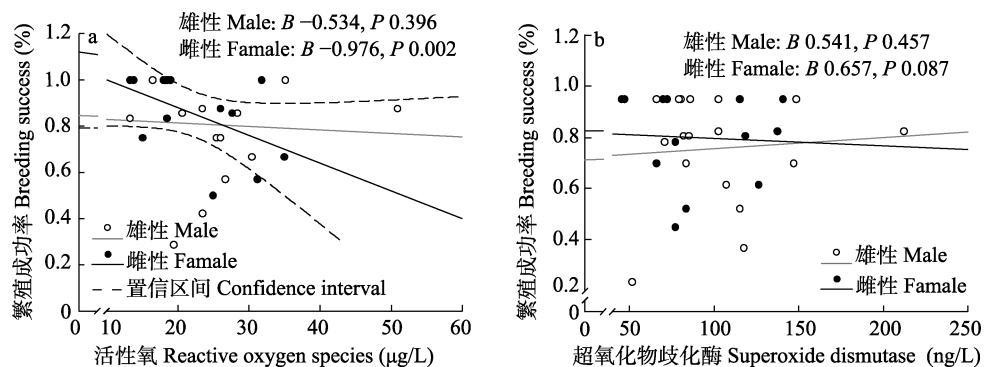

图 2 杂色山雀活性氧、超氧化物歧化酶与繁殖成功率的关系

Fig. 2 The relationship between reactive oxygen species and breeding success, and between superoxide dismutase and breeding success of Varied Tit

化酶水平与繁殖成功率无显著相关性（$B = 0.657$，$t = 1.863$，$n = 13$，$P = 0.087$，图 2b）。雄性杂色山雀活性氧、超氧化物歧化酶水平与繁殖成功率无显著相关性（活性氧：$B = -0.534$，$t = -0.872$，$n = 17$，$P = 0.396$，图 2a；超氧化物歧化酶：$B = 0.541$，$t = 0.730$，$n = 17$，$P = 0.475$，图 2b）。因此，活性氧能较好地预测雌性（而非雄性）杂色山雀的繁殖成效，而超氧化物歧化酶对雌性和雄性杂色山雀繁殖成效没有显著的预测效果。

3 讨论

作为一种综合性的生理因子，个体氧化应激水平是其身体质量和对环境适应性的综合体现。近年来，氧化应激对繁殖的预测作用受到广泛关注（Costantini et al. 2015b）。本研究分析了代表个体氧化应激水平的血浆分子活性氧和超氧化物歧化酶浓度对杂色山雀繁殖成效的预测作用。活性氧是氧化还原反应的副产物，高水平的活性氧是生活史的限制因素（Dowling 2009）。本研究结果显示，雌性杂色山雀血浆活性氧含量与其出飞率、繁殖成功率呈显著负相关，具有更低活性氧水平的雌性杂色山雀繁殖成功率及其繁殖巢幼鸟出飞率更高。超氧化物歧化酶是抗氧化防御网络的第一层次，是细胞/个体适应各种压力条件的主要驱动力（Surai 2016）。本研究并没有发现超氧化物歧化酶与杂色山雀繁殖成效间显著的相关性，这可能是由于机体内存在多种抗氧化酶及非酶促抗氧化作用，在预测繁殖成效时可能需综合考虑多种抗氧化剂的作用。

本研究结果显示，雄性杂色山雀血浆氧化应激分子活性氧和超氧化物歧化酶含量与其雏鸟出飞率及繁殖成功率均无显著相关性。这可能与杂色山雀种群繁殖策略有关，雌鸟承担主要的繁殖压力，因此氧化应激水平与繁殖成效之间存在较强的相关性，而雄鸟繁殖压力较低，其个体质量对繁殖成效的预测作用相对较弱。此外，杂色山雀对氧化应激的敏感性也可能存在性别差异，雌性个体对于氧化应激的敏感性可能更强，尤其是在生殖方面（Costantini 2018）。这种差别也见于其他鸟类种群，如雌性高山雨燕（*Tachymarptls melba*）的抗氧化能力与卵孵化率呈正相关，而雄性抗氧化能力与卵孵化率无关（Bize et al. 2008）。红腿石鸡（*Alectoris rufa*）的卵孵化率与雌性的脂质过氧化水平呈负相关，而与雄性无关（Alonso-Alvarez et al. 2010）。总的来说，本研究证实，杂色山雀雌性亲鸟血浆活性氧水平在一定程度上可以预测其繁殖成效。

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