UNIVERSITÉ DU QUÉBEC À MONTRÉAL

RELATIONS ENTRE LE PATRON D'EXPLORATION, LA REPRODUCTION ET LE NIVEAU DE CORTISOL FÉCAL CHEZ LE TAMIA RAYÉ (*TAMIAS STRIATUS*)

THÈSE

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RÉSUMÉ

Les organismes montrent des variations de cycle de vie, de comportement et de physiologie. L'écologie évolutive cherche à les expliquer en investiguant les conséquences de ces variations pour l'écologie et l'aptitude phénotypique des animaux. Ainsi, certaines populations comportent des individus ayant un cycle de vie plus rapide que d'autres, caractérisé par une reproduction plus précoce, une fécondité plus élevée mais également une longévité plus faible. Une hypothèse récente, l'hypothèse du train de vie, prédit que ces différences de cycle de vie seraient associées à des différences de comportement constantes entre les individus lorsque ces traits sont impliqués dans les compromis évolutifs. Selon cette hypothèse, les individus investissant plus dans les comportements exploratoires devraient ainsi atteindre leur succès reproducteur maximum plus tard dans leur vie. Ceci peut provenir, d'une part, du fait que le train de vie des individus détermine leur personnalité; ou d'autre part, que la personnalité d'un individu détermine comment il complétera son cycle de vie. Il est possible de gagner certains indices sur les relations entre les traits comportementaux et biodémographiques à travers l'analyse des mécanismes de régulation qui leur sont commun, comme les gluco-corticoïdes (GC). Le but général de cette thèse de doctorat est de mieux comprendre les différences de patron d'exploration en testant l'hypothèse du train de vie. Selon cette hypothèse, les individus investissant le plus dans l'exploration de leur environnement devraient montrer un succès reproducteur maximal plus tardif et mobiliser plus de GC pour protéger leur survie face aux perturbations environnementales. J'analyse ainsi les relations entre le patron d'exploration exprimé par les individus dans un environnement nouveau, leur patron de reproduction au cours de leur vie, et le niveau de cortisol (le GC principal chez les mammifères) dans une population naturelle de tamias rayés (*Tamias striatus*) située au sud du Québec dans les monts Sutton. Je valide dans un premier temps une méthode permettant le suivi du niveau de cortisol de manière non-invasive. Je documente ensuite les relations entre le patron d'exploration des individus, leur docilité lors de manipulations sur le terrain et leur propension à être capturés dans les pièges. Je détermine la relation entre le patron d'exploration des individus et leur réactivité physiologique aux perturbations environnementales. Je documente ensuite le patron de reproduction des individus tout au long de leur vie et analyse sa relation avec leur patron d'exploration. Enfin, j'analyse le niveau de cortisol des individus en nature.

Les individus ont été suivis sur 25 hectares de forêt décidue mature. Chaque été de 2005 à 2010 les individus ont été suivis par piégeage, observations focales et télémétrie. Les jeunes ont été capturés à l'émergence du terrier maternel, et assignés à leur père le plus probable à l'aide de marqueurs microsatellites. Les individus ont été soumis à des tests d'arène, quantifiant la réponse comportementale à un environnement nouveau.

Durant ce test, certains individus expriment un patron d'exploration superficiel, caractérisé par une exploration importante en début de test mais une diminution tout aussi importante de l'exploration au court des secondes suivantes. À l'autre extrême, d'autres individus expriment un patron d'exploration plus méticuleux, avec un niveau d'exploration modéré mais constant au cours de la durée du test. Les animaux ont également été soumis à des tests de docilité, lors desquels nous avons relevé le nombre de secondes passées par l'animal à se débattre durant une minute lors des manipulations. Lors des captures en 2009, des échantillons fécaux ont été prélevés dans les trappes. La concentration de métabolites issus du cortisol a ensuite été analysée par ELISA compétitive en utilisant un anticorps polyclonal. La réactivité du système sympathique des individus a été quantifiée par l'analyse du rythme cardiaque lors d'un test de restriction.

Les individus de la population d'étude montrant un patron d'exploration plus superficiel dans l'arène sont moins dociles. Ces individus sont également capturés plus fréquemment (mâles) ou plus loin de leur terrier (femelles). Les individus exprimant un patron d'exploration superficiel dans l'arène démontrent une réactivité du système sympathique plus importante, mais une variabilité plus faible de leur niveau de cortisol, suggérant qu'ils protègent ainsi leur survie de manière moins importante. En accord avec les prédictions, les individus plus superficiels dans leur exploration atteignent également leur succès reproducteur maximal plus tôt au cours de leur vie reproductive. Les variations d'abondance de nourriture d'une année à l'autre, à travers leurs effets sur l'âge à la première reproduction des individus contribuent à favoriser un meilleur succès reproducteur à vie des explorateurs superficiels parmi les individus nés durant les années de forte abondance de nourriture. À l'inverse, les explorateurs méticuleux ont un succès reproducteur plus élevé parmi les individus nés durant les années de faible abondance de nourriture. Les femelles ayant un patron d'exploration plus superficiel démontrent une variabilité plus faible de leur niveau de cortisol. Les femelles amenant un plus grand nombre de jeunes au sevrage ont également une variabilité en cortisol plus faible au cours de l'été.

Ces résultats sont en accord avec l'hypothèse du train de vie et démontrent que le patron d'exploration exprimé par les animaux est associé à leur patron de reproduction en fonction de l'âge. De plus ils suggèrent que les fluctuactions d'abondance des ressources d'une année à l'autre sont susceptibles de générer des pressions de sélection oscillantes qui pourraient maintenir la variabilité du patron d'exploration observée dans cette population. L'analyse du patron de cortisol des individus suggère enfin que le patron de reproduction et le patron d'exploration sont susceptibles de s'influencer l'un l'autre. Ces résultats contribuent à la compréhension des différences de personnalité par des résultats empiriques sur les relations entre les traits de personnalité, la physiologie et la biodémographie des individus. De telles études sont encore rares. Des analyses futures, détaillant d'une part la nature des coûts et des bénéfices associés au patron d'exploration des individus, et d'autre part investiguant les sources de variation contribuant à la variabilité du patron d'exploration observées dans cette population permettront de compléter les résultats présentés dans cette thèse.

CHAPITRE I

INTRODUCTION

1.1 Les relations entre les traits biodémographiques et comportementaux

Les organismes montrent des variations de cycle de vie, de comportement et de physiologie. Cette variation phénotypique a une double signification sur le plan évolutif. D'un coté, elle rend un certain tri sélectif possible pour éventuellement permettre l'évolution biologique (si cette variation est associée à une variation génétique). D'un autre coté, cette variation est adaptative, car elle est en partie le produit des pressions de l'évolution passée (Darwin, 1859). Dans une perspective évolutive, expliquer la signification de cette variation revient ainsi à détailler comment les traits biodémographiques, comportementaux ou physiologiques d'un organisme lui permettent de trouver et d'acquérir sa nourriture, d'éviter les prédateurs et les parasites, de croître, survivre et se reproduire (Fox, Roff et Fairbairn, 2001). Autrement dit, on cherche à comprendre comment les caractéristiques de cet organisme influencent son écologie et éventuellement son succès reproducteur (Fox, Roff et Fairbairn, 2001). Pour comprendre ceci, il est aussi nécessaire de tenir compte du fait que pour croître, survivre et se reproduire, les organismes disposent de ressources en quantité limitée. Ceci génère des contraintes puisque tout investissement de temps, d'énergie ou de nutriments dans un trait, est réalisé au détriment d'un autre trait (Stearns, 1992; Roff et Fairbairn, 2007), ce qui empêche l'expression de certaines combinaisons de traits par les organismes (Perrin et Travis, 1992). Par exemple, il est impossible pour un individu donné de produire le nombre maximal de jeunes tout en ayant la longévité la plus importante. Le principal compromis concerne l'investissement

des organismes dans leur reproduction immédiate par rapport à l'investissement réalisé dans leur survie et leur reproduction future (Williams, 1966; Stearns, 1992).

1.2 L'hypothèse du syndrome de train de vie

Parce qu'ils font face à des compromis, les organismes sont contraints de 'prendre des décisions' d'allocation ou de 'choisir', c'est à dire d'investir leurs ressources à exprimer certaines caractéristiques plutôt que d'autres (Brockmann, 2001; Brockmann et Taborsky, 2008). Par exemple, lorsqu'un animal investit ses ressources dans les organes et les caractéristiques permettant de se reproduire plus tôt, ces ressources ne sont plus disponibles pour les fonctions de l'organisme associées à une reproduction plus tardive ou à une survie plus élevée. Les décisions d'allocation touchent généralement plus d'un trait et s'appuient sur des mécanismes physiologiques multiples qui ont une flexibilité limitée au cours de la vie de l'animal, par exemple la taille des organes digestifs ou du foie (Stamps, 2007; Biro et Stamps, 2008; Wiersma, Nowak et Williams, 2012). Ainsi, de telles décisions d'allocation sont contraignantes pour les animaux. Ces décisions d'allocation sont également exprimées en réponse à certaines conditions environnementales, comme par exemple l'abondance de nourriture, ou la densité de la population. Ainsi les individus favorisant leur reproduction immédiate par rapport à leur reproduction future sont généralement plus aptes dans des situations de forte abondance de nourriture (Boon, Réale et Boutin, 2007; Dingemanse et de Goede, 2004). À l'inverse, les individus dont l'environnement ne permet pas une reproduction dans le futur devraient allouer la majorité de leurs ressources à leur reproduction immédiate (Stearns, 1992).

Ainsi, les compromis évolutifs, en interaction avec les conditions environnementales amènent l'évolution de suites d'adaptations, ou stratégies alternatives, accordant une importance différente aux composantes de leur aptitude phénotypique (Stearns, 1992). De telles stratégies peuvent regrouper à la fois des traits biodémographiques, comportementaux et physiologiques. Ces différentes facettes de la stratégie d'un individu sont souvent décrites comme formant son train de vie (Ricklefs et Wikelski, 2002). Par exemple, dans certaines populations, on retrouve des individus ayant un cycle de vie plus

rapide que d'autres, caractérisé par une reproduction plus précoce, une fécondité plus élevée mais également une longévité plus faible (Gaillard et al., 1989; Promislow et Harvey, 1990; Bielby et al., 2007, voir figure 1.1 a). Le train de vie regroupe non seulement des traits biodémographiques, mais également des différences de comportement (Wolf et al., 2007; Wolf, van Doorn et Weissing, 2008). En particulier, les individus ayant un cycle de vie rapide, devraient exprimer des comportements permettant d'acquérir des ressources rapidement même si cette acquisition implique un risque de mortalité pour les animaux (Wolf et al., 2007; Biro et Stamps, 2008). Par exemple, les individus ayant un train de vie rapide devraient être plus hardis, et continuer à s'approvisionner dans des situations où un risque de prédation est présent. Ils devraient également être plus agressifs de manière à défendre leurs ressources (territoire, partenaires sexuels, nourriture), ou encore plus actifs et explorateurs, lorsque ces comportements sont associés à l'acquisition de nourriture (Biro et Stamps, 2008; Réale et al., 2010a). Alternativement, il est aussi possible que l'exploration interviennent dans les compromis évolutifs à travers ses coûts, qui sont immédiats, et ses bénéfices qui sont généralement disponibles dans le future. Par exemple, les animaux qui investissent plus dans l'exploration de leur environnement le font au détriment d'autres comportements permettant d'acquérir des ressources immédiatement (Stamps, 2007). Par contre, l'information qu'ils retirent de leur exploration devrait être bénéfique à long terme, en leur permettant de sélectionner un meilleur habitat, de mieux prédire la disponibilité de nourriture dans le future, ou encore d'échapper aux prédateurs plus efficacement (Elliott, 1978; Mangel et Stamps, 2001; Wolf et al., 2007). Sous cette prémisse, l'exploration serait associée, non pas à la survie des individus, mais à leur fécondité au long de leur vie (Biro et Stamps, 2008). Les individus investissant plus dans les comportements exploratoires devraient ainsi atteindre leur succès reproducteur maximum plus tard dans leur vie (voir figure 1.1 b).

Les relations entre les traits comportementaux et les traits biodémographiques peuvent provenir de deux mécanismes distincts. D'une part, les décisions d'allocation des ressources à tel ou tel trait biodémographique modifient et déterminent le comportement des animaux (Wolf et al., 2007). Par exemple, les animaux chez lesquels on réduit les

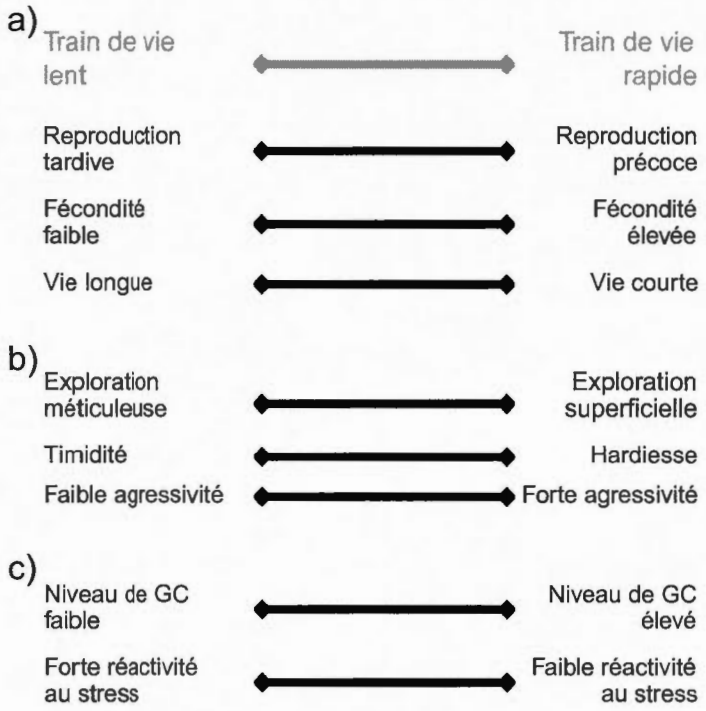

Figure 1.1 Relations entre les traits biodémographiques, comportementaux et physiologiques prédites par l'hypothèse du syndrome de train de vie. a) Les compromis évolutifs entre différentes composantes du succès reproducteur (en gris) génèrent des suites d'adaptations au niveau des traits biodémographiques, tels que l'âge à la première reproduction et la fécondité. Les individus peuvent ainsi exprimer des stratégies biodémographiques différentes. b) Les traits comportementaux, tels que l'exploration, la hardiesse ou l'agressivité, devraient co-évoluer avec les stratégies biodémographiques, lorsque ces traits sont impliqués dans les mêmes compromis évolutifs. c) De telles relations entre la stratégie biodémographique et le comportement des individus pourraient également co-évoluer avec les caractéristiques physiologiques des individus, comme par exemple leur métabolisme, ou encore la quantité d'énergie qu'ils mobilisent pour protéger leur survie lorsque les conditions environnementales sont perturbées. Ces différences physiologiques sont également contraignantes pour les organismes et devraient imposer une certaine constance aux différences individuelles de train de vie. Ces traits physiologiques peuvent être étudiés à travers l'analyse du niveau moyen de gluco-corticoïdes (GC) et de la réactivité au stress (figure adaptée de Réale et al., 2010a).

chances de survie, changent leur niveau de hardiesse et d'exploration (Nicolaus et al., 2012). D'autre part, il est possible que le comportement d'un individu détermine comment il complétera son cycle de vie, par ses effets sur la quantité de nourriture acquise, ou encore les risques de mortalité associés avec l'expression de ces comportements (Stamps, 2007). Répondre à ces questions nécessite bien entendu une approche expérimentale, manipulant les caractéristiques du cycle de vie ou le comportement des individus pour déterminer les effets d'un trait sur les autres. De telles études sont rares (Nicolaus et al., 2012). Alternativement, il est possible de gagner certains indices sur les relations entre les traits comportementaux et biodémographiques à travers l'analyse des mécanismes de régulation qui leur sont commun. D'un point de vue évolutif, comprendre la nature des relations entre ces deux types de traits est important puisque la nature et l'étroitesse de ces relations affecte comment ils répondront à d'éventuelles pressions de sélection (McGlothlin et Ketterson, 2008).

1.3 Le rôle des gluco-corticoïdes dans le train de vie

Les traits biodémographiques et comportementaux sont régulés par les mêmes mécanismes endocriniens. En particulier, les gluco-corticoïdes, sécrétés par l'axe hypothalamo-pituitaire-surrénalien (HPS), régulent à la fois les caractéristiques biodémographiques telles que l'investissement reproducteur et la croissance des individus, ainsi que leur comportement, par exemple leur agressivité et leur comportement d'exploration. Les animaux ajustent également le niveau de gluco-corticoïdes en fonction des conditions environnementales comme l'abondance de nourriture, ou la densité des populations (Reeder et Kramer, 2005; Wingfield, 2005; McGlothlin et Ketterson, 2008). À des concentrations situées en dessous d'un certain seuil (Romero 2009), les gluco-corticoïdes permettent de mobiliser les ressources de l'organisme pour favoriser les fonctions associées à la croissance et la reproduction. Par exemple, chez l'hirondelle bicolore (*Tachycineta bicolor*) le niveau de gluco-corticoïdes des femelles durant la reproduction augmente avec la masse des jeunes qu'elles produisent (Bonier et al., 2009). Les gluco-corticoïdes augmentent également lorsque la disponibilité de nourriture est faible (Kitaysky, Wingfield et Piatt, 1999; Ki-

taysky et al., 1999; Romero et Wikelski, 2001), de manière à favoriser un plus grand niveau d'activité et des comportements d'approvisionnement (Astheimer, Buttemer et Wingfield, 1994; Gutman et al., 2011).

Lorsque leur concentration dépasse un certain seuil ou que l'environnement de l'animal est perturbé (c'est à dire lorsqu'il subit un stress), les gluco-corticoïdes ont cependant un autre effet (Wingfield et Kitaysky, 2002; Reeder et Kramer, 2005). Une perturbation peut par exemple être une attaque par un prédateur, une tempête ou encore une interaction agonistique avec un congénère. Face à une perturbation, les individus sécrètent en quelques minutes une quantité importante de gluco-corticoïdes qui inhibe les fonctions de l'organisme associées à la reproduction pour rediriger les ressources vers les fonctions assurant la survie (Reeder et Kramer, 2005). Par exemple, les bruants à gorge blanche (*Zonotrichia albicollis*) soumis à une tempête durant leur période de nidification montrent une augmentation importante du niveau de gluco-corticoïdes (Wingfield et al., 1998), qui inhibe leur comportement reproducteur, mobilise les réserves d'énergie disponibles au foie et dans les tissus adipeux vers les muscles en vue d'un effort pour quitter le site de reproduction (Wingfield, 2005). Chez la plupart des espèces, cette réponse est également accompagnée d'une augmentation des capacités cognitives (Breuner, Wingfield et Romero, 1999; Reeder et Kramer, 2005). La réactivité au stress semble donc associée à l'importance que les animaux accordent à leur survie et leur reproduction future (Wingfield, 2005; Breuner, Patterson et Hahn, 2008; Bonier et al., 2009). Elle est plus importante chez les espèces montrant un train de vie lent (Wiersma, Munoz-Garcia et Williams, 2007). À l'inverse, elle est diminuée ou cesse d'exister durant la reproduction chez les espèces qui ont des chances de reproduction future limitées ou chez lesquelles les individus font face à une compétition intense pour les partenaires sexuels (Boonstra, 2005; Bókony et al., 2009). À l'intérieur d'une population, les individus peuvent également moduler leur réactivité au stress en fonction de leur effort parental, de la valeur de leur investissement reproducteur ou encore de leur âge. Ainsi, chez les oiseaux où les mâles et les femelles expriment des soins parentaux, le sexe contribuant le plus aux soins montre une réactivité diminuée face au stress (O'Reilly

et Wingfield, 2001; Holberton et Wingfield, 2003). Les individus de certaines populations d'oiseaux montrent également une diminution de leur réactivité au stress plus importante lorsqu'ils s'occupent de portées plus nombreuses ou plus lourdes (Lendvai et Chastel, 2008). Enfin, les individus diminuent leur réactivité au stress au fur et à mesure qu'ils vieillissent et que les chances de reproduction future diminuent (Heidinger, Nisbet et Ketterson, 2006; Angelier et al., 2007a). Ces études prédisent que les individus accordant plus d'importance à leur survie et leur reproduction future qu'à leur reproduction immédiate devraient produire plus de cortisol face à une perturbation environnementale donnée.

La réactivité au stress détermine également le niveau d'agressivité des animaux face à leurs congénères, leur niveau de hardiesse face à un risque de prédation ou encore leur patron d'exploration dans un environnement inconnu (Benus et al., 1989; Koolhaas et al., 1999; Carere et al., 2005; Overli et al., 2007). Ainsi les animaux produisant plus de cortisol en réponse à un risque de prédation ou dans un environnement nouveau ont tendance à être plus timides et moins explorateurs ou moins actifs (Koolhaas et al., 1999; Carere et al., 2005; Cockrem, 2007; Overli et al., 2007; Atwell et al., 2012). Des expériences de sélection artificielle sur la réactivité au stress amènent des changements corrélés au niveau de l'activité, de l'agressivité et de la hardiesse des individus (Overli, Kotzian et Winberg, 2002; Overli, Winberg et Pottinger, 2005; Schjolden et al., 2005). Les relations entre le train de vie et les traits de personnalité pourraient ainsi provenir des différences individuelles de niveau moyen de gluco-corticoïdes, associées à l'équilibre énergétique des individus, et de réactivité aux perturbations environnementales, protégeant leur survie (voir figure 1.1c). D'une part, il est possible que les différences individuelles de réactivité au stress associées à la personnalité des individus contraignent la capacité des individus à se reproduire et déterminent leur âge à la première reproduction et leur fécondité. D'autre part, il est également possible que les décisions de reproduction des individus, en affectant leur réactivité au stress, modifient leur comportement. Départager ces deux scénarios améliorerait grandement notre compréhension des relations entre les traits de personnalité et le train de vie des individus. Ceci nécessite cependant d'analyser simul-

tanément les relations entre la réactivité au stress et la reproduction des individus d'une part, et entre la réactivité au stress et la personnalité des individus d'autre part. Si le patron de reproduction des individus est contraint par leur réactivité au stress, associées à la personnalité, le succès reproducteur des individus ne devrait pas montrer de relation avec le niveau de cortisol une fois ces différences individuelles de personnalité prises en compte. À l'inverse, si le patron de reproduction des individus affecte leur personnalité, on s'attend à ce que la reproduction soit associée au patron de cortisol même après avoir pris en compte la personnalité des individus. Il est également possible que des individus montrant des tactiques de reproduction ou des trains de vie différents réagissent différemment à une augmentation ou à une diminution de gluco-corticoïdes (Lancaster et al., 2008). Par exemple, chez le lézard (*Uta stansburiana*), les femelles qui expriment des stratégies alternatives répondent à une augmentation de gluco-corticoïdes soit par un investissement reproducteur plus important, soit par une inhibition de la reproduction (Lancaster et al., 2008). Même si beaucoup d'études ont mesuré les relations entre le niveau de gluco-corticoïdes, les traits biodémographiques et le comportement, il est encore très rare d'appliquer ce genre de suivi au niveau individuel et à long terme. De plus, aucune étude n'a, à ma connaissance, étudié simultanément le niveau moyen de cortisol et la réactivité au stress. Ceci est réalisable en utilisant des mesures non-invasives, permettant de quantifier le niveau de gluco-corticoïdes de manière répétée. Il est par exemple possible de doser la quantité de métabolites des gluco-corticoïdes naturellement présents dans l'urine et dans les fèces (Palme et al., 2005), ou les poils et les plumes (Bortolotti et al., 2008; Martin et Réale, 2008). Ces méthodes produisent une mesure intégrée de la quantité de gluco-corticoïdes sécrétée au cours d'une période allant de quelques heures, dans l'urine ou les fèces, (Dantzer et al., 2010) à plusieurs jours, dans les poils ou plumes, (Bortolotti et al., 2008) L'utilisation de ces mesures demande cependant une validation appropriée de la technique sur l'espèce étudiée, afin de s'assurer que les métabolites dosés dans les fèces représente bien la quantité de gluco-corticoïdes sécrétées dans le sang de l'animal. Une telle validation permet également de déterminer le temps mis par les gluco-corticoïdes entre leur sécrétion dans le sang et leur excrétion sous forme de métabolites (Palme, 2005).

En résumé, le but général de cette thèse de doctorat est de mieux comprendre les différences de train de vie que les animaux expriment, en déterminant comment ces différences sont maintenues sur le plan évolutif. Pour ce faire, je documenterai dans un premier temps le patron de reproduction, et le comportement des individus tout au long de leur vie (van de Pol et Verhulst, 2006). Deuxièmement, je déterminerai les conséquences du comportement des individus pour leur succès reproducteur. Enfin, afin de mieux comprendre comment l'expression de chacun des traits faisant partie des syndromes de train de vie affecte l'expression des autres traits qui y sont associés, j'analyserai le niveau de gluco-corticoïdes des individus en nature et en relation à leur reproduction et leur personnalité. À cette fin, je validerai une approche permettant de mesurer de manière non-invasive le niveau de gluco-corticoïdes des individus en nature, et de distinguer les fluctuations saisonnières associées à l'état énergétique des individus de leur réponse aux perturbations environnementales. J'utiliserai le tamia rayé (*Tamias striatus*) comme modèle d'étude.

1.4 Modèle d'étude

Le tamia rayé (*Tamias striatus*) est un Sciuridé de l'Est de l'Amérique du nord. On le retrouve principalement dans les forêts décidues relativement matures (Snyder, 1982). Le tamia dépend en majeure partie de noix et graines relâchées par des arbres à paîsson pour se nourrir. L'abondance de la nourriture disponible varie donc considérablement d'une année à l'autre (Elliott, 1978; Munro, Thomas et Humphries, 2008) et ceci détermine en majeur partie le recrutement de la population et la survie des individus (Bergeron et al., 2011a). Le tamia rayé emmagasine des glands et noix dans son terrier pour l'hiver (Elliott, 1978). À cette saison, il peut entrer en torpeur (French, 2000; Landry-Cuerrier et al., 2008). Le tamia rayé vit en moyenne 2 à 3 ans, mais de rares individus peuvent atteindre 7 ans (Snyder, 1982). Il se reproduit une à deux fois par année (Elliott, 1978; Bergeron et al., 2011a) selon la présence d'un mast. Ainsi, les années où les hêtres (*Fafus grandifolia*) relâchent une grande quantité de graines, la saison d'accouplement a lieu en été de manière à ce que les jeunes émergent et se dispersent à l'apogée de

l'abondance de nourriture (Bergeron et al., 2011a). Les adultes sont également en mesure de stocker une quantité de nourriture leur permettant non seulement de passer l'hiver, mais également de se reproduire de nouveau au printemps suivant (Bergeron et al., 2011a). Ceci donne lieu à un espacement irrégulier des saisons de reproduction, qui contraint l'histoire de vie des individus selon le moment où ils naissent (voir figure 1.2).

Les saisons de reproduction ayant lieu en été ou au printemps (Elliott, 1978) présentent des conditions contrastées (Bergeron et al., 2011b). En été, les accouplements s'étalent sur une période de 3 semaines. Durant cette période, les mâles augmentent l'espace qu'ils utilisent, pour visiter les femelles avoisinantes et ainsi sonder leur réceptivité. L'oestrus des femelles ne dure qu'une journée, lors de laquelle plusieurs mâles se regroupent sur le domaine vital de la femelle et tentent de copuler avec elle, donnant lieu à des chasses reproductives (Elliott, 1978; Schulte-Hostedde et Millar, 2002). Puisque la plupart des adultes participent à la reproduction d'été, on peut voir jusqu'à 10 mâles pourchasser une femelle donnée (Elliott, 1978). La complexité du sous bois et la forte densité de mâles induisent une mêlée importante. Certains mâles tentent cependant de monopoliser l'accès aux femelles par des interactions agressives (Elliott, 1978), bien que cela n'aie jamais été quantifié. À l'inverse, durant les reproductions de printemps, on n'observe généralement qu'un ou deux mâles sur le territoire d'une femelle lors de son oestrus. La femelle passe la majorité de son oestrus dans son terrier. Il est donc possible pour un mâle de garder l'entrée du terrier d'une femelle et de copuler avec elle de manière répétée. Le contraste entre les saisons de reproduction estivales et printanières se traduit également par une grande variation au niveau du taux de paternité multiple. Tandis qu'à l'été chaque jeune issu d'une portée donnée est issu d'un père différent, au printemps, la plupart des jeunes issus d'une même portée sont produits par un seul mâle (Bergeron et al., 2011b).

La période de gestation est d'environ 30 jours (Snyder, 1982). La femelle donne naissance à une portée de 4 à 6 jeunes en moyenne (Pidduck et Falls, 1973). Les jeunes passent le premier mois de leur vie dans le terrier maternel avant de se disperser (Elliott, 1978).

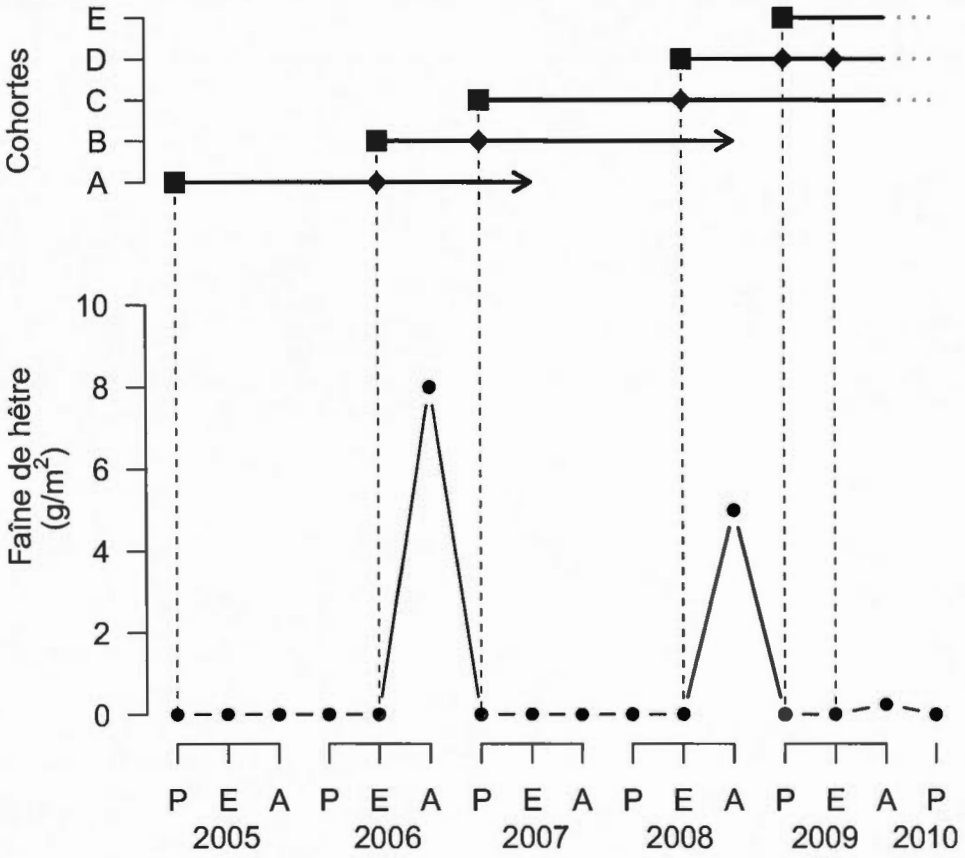

Figure 1.2 Phénologie des saisons de reproduction observées chez les tamias rayés des monts Sutton de 2005 à 2010 (carrés noirs), en fonction de la quantité de graines relachées par les hêtres de l'aire d'étude (trait plein et points noirs). Les saisons de reproduction peuvent se produire au printemps (P) ou en été (E). Les masts se sont produits en automne (A) 2006 et 2008. Les individus nés en 2005 (cohorte A) et en 2007 (cohorte C) sont nés au printemps. Les individus nés en 2006 et 2008 sont nés en été. La première opportunité de reproduction (losanges noirs) est de 15 mois pour les cohortes A et C, et de 7 mois pour la cohorte B. Les mâles de la cohorte D ont eu une première opportunité de reproduction à 7 mois, et les femelles à 10 mois.

On constate que les jeunes nés en été émergent du terrier maternel et se dispersent à des poids beaucoup plus légers (40 g en moyenne) que les jeunes nés au printemps (60 g en moyenne). Chez cette espèce, la dispersion varie selon le sexe des individus, les mâles se dispersant sur de plus grandes distances que les femelles (Loew, 1999; Dubuc Messier, Bergeron et Réale, 2012).

1.4.1 Suivi de la population

L'aire d'étude consiste en une grille carrée de 25 hectares située dans une forêt décidue mature où l'érable à sucre (*Acer Saccharum*) et le hêtre (*Fagus grandifolia*) dominent. Elle est localisée dans le Sud du Québec près des monts Sutton (45°05'N; 72°26'O). Chaque été de 2005 à 2010 les animaux ont été suivis par piégeage, observations focales et télémétrie. Une grille de piégeage de 255 trappes Longworth a été utilisée pour capturer les individus (Voir figure 1.3). Les trappes étaient disposées tous les 40 mètres sur 26 lignes, de manière à couvrir la majorité de l'aire d'étude. Les captures effectuées sur cette grille de piégeage ont été complémentées par des captures réalisées en périphérie de la grille et des captures ciblant spécifiquement certains terriers, permettant de capturer la quasi-totalité des individus présents sur le site d'étude (Bergeron et al., 2011a). Les pièges étaient ouverts le matin, inspectés toutes les 2h et fermés à la tombée de la nuit. Chaque individu de la population a été marqué avec des bagues métalliques (National Band & Tags Co., New York, KY) et un transpondeur unique sous-cutané (Eidap Inc., Alberta, Canada). Le sexe, la masse, le statut reproducteur des individus et la présence de parasites ont été relevés lors de chaque capture. Pendant l'été, les individus étaient équipés de colliers émetteurs pesant un maximum de 4 g (model PD-2C, Holohil Systems Ltd, Ontario), permettant de localiser leur terrier. Les jeunes ont été capturés à l'émergence du terrier maternel lors de chaque reproduction, afin de les marquer, et de prélever un échantillon de tissu. Ces échantillons ont été utilisés pour des analyses moléculaires à l'aide de marqueurs microsatellites permettant d'assigner les jeunes à leur père le plus probable (Chambers et Garant, 2010; Bergeron et al., 2011b).

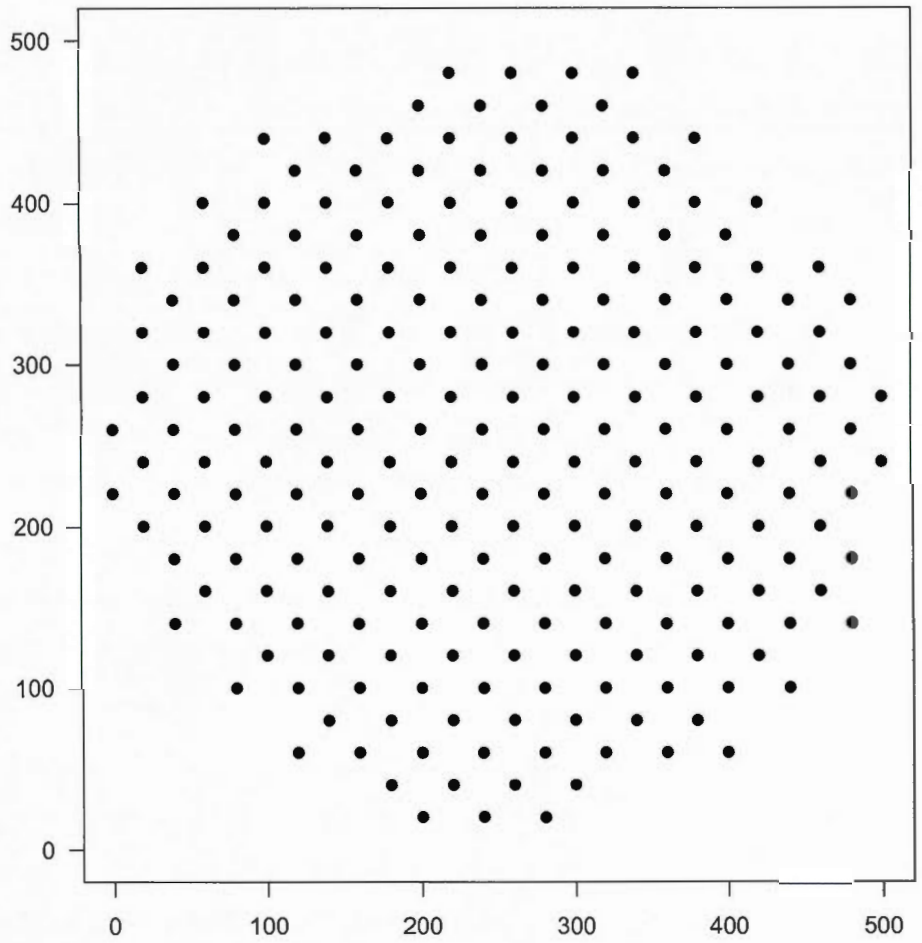

Figure 1.3 Grille de piégeage utilisée pour réaliser le suivi de la population d'étude (500 X 500 m). La grille est composée de 26 lignes de pièges Longworth (points noirs) disposés en quinconce tous les 40 mètres. La totalité de la grille a été piégée au moins une fois par semaine chaque année du mois d'avril au mois d'octobre. Durant les séances de piégeage, les pièges étaient armés le matin vers 0800 h, inspectés toutes les 2 h et fermés au coucher du soleil.

1.4.2 Tests comportementaux

De 2006 à 2010, nous avons également conduit des tests comportementaux sur les individus de la population. La plupart des individus ont été soumis à un ou deux tests d'arène. Le test d'arène est utilisé couramment pour quantifier le comportement d'exploration chez les rongeurs en laboratoire (Archer, 1973; Archer, 1975). Ce test a également été utilisé avec succès en nature pour quantifier le patron d'exploration chez le tamia rayé (Martin et Réale, 2008). Ce test consiste à placer l'individu dans un environnement nouveau et à quantifier sa réponse comportementale durant 90 secondes. Ces tests ont été réalisés directement sur le site d'étude, afin de minimiser les perturbations causées par l'hébergement en captivité ou le transport à un laboratoire. La plupart des individus ont été testés une première fois durant leur première année. Lorsque ceci a été possible, nous avons testé les individus une seconde fois l'année suivante. J'ai déjà montré que le comportement d'un individu durant ce test est répétable au cours de sa vie, et que les individus diffèrent de manière constante au niveau du patron d'exploration qu'ils expriment dans l'arène (Montiglio et al., 2010). À un extrême, certains individus expriment un patron d'exploration superficiel, caractérisé par une exploration importante en début de test mais une diminution tout aussi importante de l'exploration durant les secondes suivantes ; tandis qu'à l'autre extrême, d'autre individus expriment un patron d'exploration plus méticuleux, avec un niveau d'exploration modéré mais constant au cours de la durée du test (Montiglio et al., 2010, voir figure 1.4). Ces patrons d'exploration suggèrent que, bien que les individus superficiels expriment un niveau d'exploration plus élevé durant le test, les individus ayant un patron plus méticuleux devraient investir plus de temps et d'énergie dans l'exploration de leur environnement à long terme, puisque leur niveau d'exploration est soutenu dans le temps (Montiglio et al., 2010). Lors de chaque capture, les animaux ont également été soumis à un test de docilité. Lors de ce test, les animaux ont été transférés de la trappe à un sac de manipulation. Nous avons ensuite relevé le nombre de secondes passées par l'animal à se débattre durant une minute.

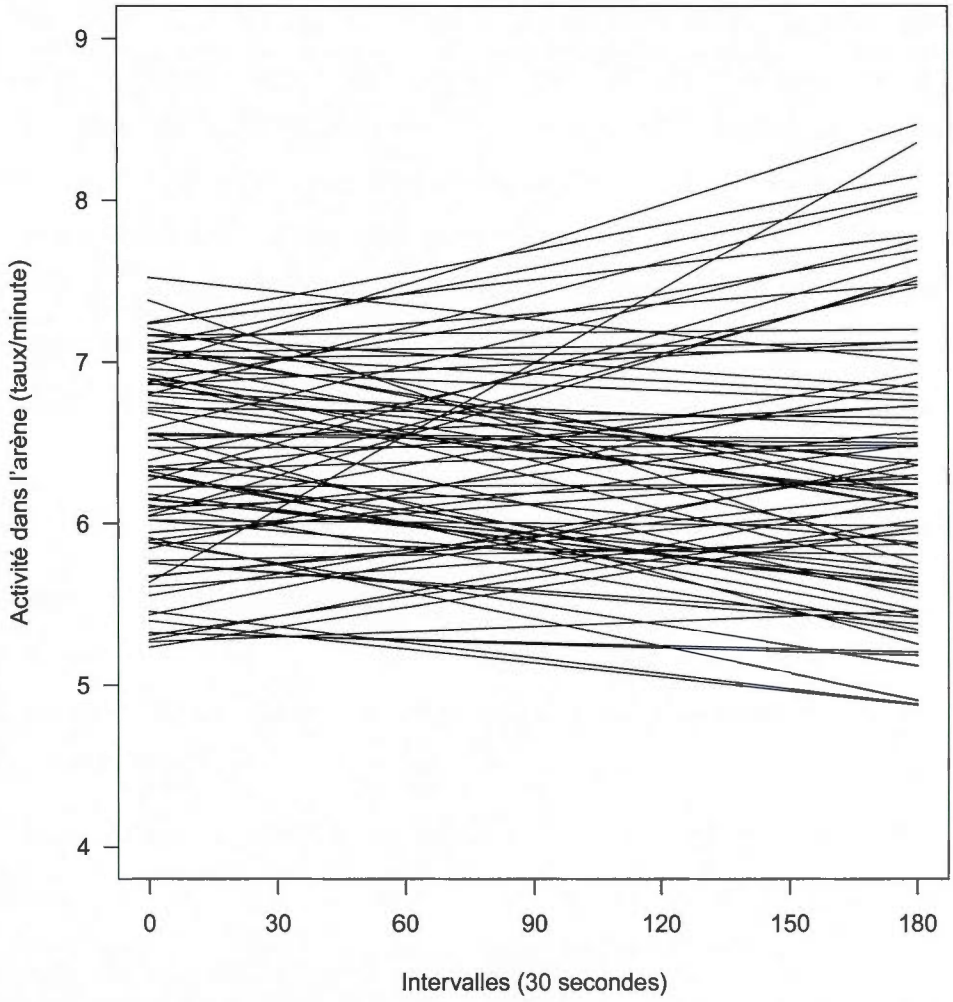

Figure 1.4 Patrons d'exploration exprimés par les individus chez le tamia rayé dans les monts Sutton en fonction des trois intervalles successifs de 30 secondes consituant le test d'arêne, tiré de Montiglio et al., 2010.

1.4.3 Mesure du niveau de cortisol fécal

Lors de la plupart des captures en 2009, des échantillons fécaux ont été prélevés dans les trappes. Les échantillons ont été mis sur glace pour le reste de la journée avant d'être transférés à - 20 °C. Les échantillons ont ensuite été entreposés à - 80 °C moins de deux semaines après leur collecte. Les échantillons ont été séchés jusqu'à l'obtention d'une masse constante à 70 °C avant d'être préparés pour le dosage des métabolites du cortisol (voir chapitre I). Ils ont été pulvérisés à l'aide d'un mortier. Une quantité de la matière fécale (35 ± 5 mg) a ensuite été vortexée dans 1 ml de méthanol 80 % puis centrifugée. La concentration de métabolites du cortisol a ensuite été analysée par ELISA compétitive en utilisant un anticorps polyclonal ciblant le cortisol (voir chapitre I).

1.5 Structure de la thèse, objectifs et prédictions

L'objectif général de cette thèse est de mieux comprendre les stratégies de train de vie exprimées par les individus dans la population d'étude. Je présente tout d'abord les aspects techniques validant l'utilisation d'un essai immunologique pour quantifier le niveau de métabolites du cortisol dans les fèces des tamias. Cette étude constitue le chapitre II.

Je décris dans un second temps les relations entre le patron d'exploration, la trappabilité et la docilité afin de préciser comment le comportement d'exploration peut affecter l'écologie des individus de cette population. Je décris également les relations entre le patron d'exploration et les caractéristiques physiologiques de la réponse des individus aux perturbations environnementales. Si le patron d'exploration reflète bel et bien la réponse physiologique des individus face aux perturbations environnementales, je prédis que les explorateurs méticuleux, accordant une plus grande importance à leur aptitude phénotypique future, exprimeront une réponse de gluco-corticoïdes plus importante aux diverses perturbations de leur environnement. Ces deux aspects font l'objet du chapitre III.

J'analyse également les relations entre le patron d'exploration des individus et leur succès reproducteur. Les traits de personnalité ayant un effet sur l'acquisition des ressources et la survie des individus devraient montrer une relation avec les caractéristiques de leur cycle de vie. Ainsi, basé sur la prémisse qu'un patron d'exploration méticuleux dans l'arène est associé à une acquisition d'information bénéfique à l'aptitude phénotypique future des individus, au détriment de leur aptitude immédiate, je prédis que les explorateurs méticuleux devraient exprimer une première reproduction plus tardive. Ils devraient également obtenir leur succès reproducteur maximal plus tard au cours de leur vie reproductive. Pour déterminer si les individus ayant des patrons d'exploration différents sont favorisés par les fluctuations inter-annuelles d'abondance de nourriture, j'analyse ces relations sur plusieurs années. Si un patron d'exploration plus méticuleux est associé à un train de vie plus lent, je prédis que les individus méticuleux auront un succès reproducteur à vie plus important lorsqu'ils naissent dans des cohortes de naissance ayant un âge à la première opportunité de reproduction plus tardive. Ceci est présenté dans le chapitre IV.

Enfin, j'analyse les relations entre le niveau de cortisol, la réactivité au stress, le succès reproducteur et le patron d'exploration des individus. Si les relations entre le patron d'exploration et les traits biodémographiques des individus proviennent des contraintes exercées par leur réactivité gluco-corticoïque aux perturbations de leur environnement, je prédis que les différences individuelles de réactivité au stress devraient être associées uniquement à leur patron d'exploration. À l'inverse, si le patron de reproduction exprimé par les individus influence dans une certaine mesure leur patron d'exploration à travers ses effets sur la réactivité aux perturbations environnementales, le succès reproducteur devrait également expliquer les différences individuelles de réactivité au stress, même lorsque le patron d'exploration est pris en compte. Ces analyses sont présentées dans le chapitre V.

CHAPITRE II

NON-INVASIVE MONITORING OF FECAL CORTISOL METABOLITES IN THE EASTERN CHIPMUNK (*TAMIAS STRIATUS*) : VALIDATION AND COMPARISON OF TWO ENZYME IMMUNOASSAYS

Pierre-Olivier Montiglio, Fanie Pelletier, Rupert Palme, Dany Garant, Denis Réale & Rudy Boonstra

2.1 Abstract

Monitoring fecal glucocorticoid metabolites in wild animals, using enzyme immunoassays, enables the study of endocrinological patterns relevant to ecology and evolution. While some researchers use antibodies against the parent hormone (which is typically absent from fecal samples), others advocate the use of antibodies designed to detect glucocorticoid metabolites. We validated two assays to monitor fecal cortisol metabolites in the eastern chipmunk (*Tamias striatus*). We compared an antibody produced against cortisol and one produced against 5α-pregnane-3β, 11β, 21-triol-20-one using a radiometabolism study and an injection with adrenocorticotropic hormone (ACTH). Most cortisol metabolites were excreted in the urine ($\sim$ 83 %). Peak excretion in the feces occurred 8 h after injection. Both assays detected an increase in fecal cortisol metabolite levels after injection of ACTH. Males, but not females, exhibited a circadian variation in metabolite levels. The sexes did not exhibit any difference over the time course and route of excretion or the relative increase in fecal cortisol metabolite levels after ACTH injection. The cortisol assay displayed higher reactivity to ACTH injection

relative to baseline than did the metabolite assay. While both antibodies gave comparable results, the cortisol antibody was more sensitive to changes in plasma cortisol levels in eastern chipmunks.

2.2 Introduction

Studies of the hypothalamic-pituitary-adrenal (HPA) axis in free-ranging populations provide insights into how animals adjust their physiology, behavior, and life history to environmental perturbations (Boonstra, 2005; Reeder et Kramer, 2005; Wingfield, 2005). A better understanding of this axis sheds light on central processes in ecology and evolution, such as the longterm consequences of predation on population dynamics (Sheriff, Krebs et Boonstra, 2010; Sheriff, Krebs et Boonstra, 2011), adaptive maternal effects (Breuner, 2008; Love et Williams, 2008a), or the impact of human activities on animal populations (Thiel et al., 2008; Thiel et al., 2011; Macbeth et al., 2010).

The activity of the HPA axis is classically studied by quantifying the secretion of its end products, glucocorticoids, in the blood. The last decades have witnessed important methodological improvements to monitor glucocorticoid levels in a less invasive manner, and it is now possible to quantify their metabolites in urine and feces (Palme, 2005; Sheriff et al., 2011a). Fecal measurements may be preferable to blood measurements because they represent an integration of glucocorticoid levels in the blood over a few hours rather than the point estimates obtained from blood samples (Touma et al., 2003; Sheriff, Krebs et Boonstra, 2010). Thus, they are buffered against small fluctuations in glucocorticoid levels that are likely to occur with blood measurements and may reflect more accurately the baseline glucocorticoid level of the animal (Goymann, 2005). Moreover, recent work has shown that the fecal glucocorticoid metabolite level of an animal may be a good index of an animal's capacity to secrete plasma glucocorticoids in response to an environmental perturbation (Sheriff, Krebs et Boonstra, 2010). Concentrations of fecal glucocorticoid metabolites thus enable studying not only HPA activity but also potentially its reactivity to environmental perturbations.

Measuring glucocorticoids in their metabolized form, however, introduces some complexities. First, the time over which blood levels of glucocorticoids are integrated in the fecal samples will depend on the species' biology and activity pattern (Palme, 2005). Second, the proportion of glucocorticoid metabolites excreted via the feces or the urine may vary with the species or the sex of the animals (Touma et al., 2003; Palme et al., 2005). It is thus advisable to perform a radiometabolism study, where a known quantity of radiolabeled glucocorticoid is injected and traced in the feces as well as in the urine to assess the time lag between the secretion of glucocorticoids into the blood and the excretion of their metabolites in the feces and urine. Third, when measuring fecal glucocorticoid metabolites, one deals with a mixture of compounds displaying different molecular structures, in which little or no native (i.e., unmetabolized) glucocorticoid(s) is present (Möstl, Rettenbacher et Palme, 2005; Palme, 2005; Bosson, Palme et Boonstra, 2009). It is thus necessary to ensure that the technique used to assess glucocorticoid reacts with an important portion of the glucocorticoid metabolites produced by the animal. It is also important to ensure that glucocorticoid secretion into the blood is well reflected in the fecal samples (Touma et Palme, 2005).

Fecal glucocorticoid metabolites can be quantified with different approaches, including enzyme immunoassays (EIAs). Immunoassays originally designed to target native (i.e., unmetabolized) glucocorticoids have been extensively used to quantify fecal glucocorticoid metabolites in an array of species (Harper et Austad, 2000; Wasser et al., 2000; Young et Monfort, 2009). These assays assume that the antibody has sufficient cross-reactivity to recognize (a group of) metabolites of the parent hormone and that an increase in blood glucocorticoid secretion leads to an increase in metabolite excretion in the feces. These assays were initially developed for blood measurements (Möstl, Rettenbacher et Palme, 2005). Although these assays have different cross-reactivities and hence different efficacy, they have been used with success to study relationships between fecal glucocorticoid metabolite levels and some aspects of the reproduction or the ecology of the animals in their natural environment (Jurke et al., 1997; Lynch, Ziegler et Strier, 2002; Mateo, 2007; Young et Monfort, 2009). Other studies use antibodies designed

to recognize a group of fecal glucocorticoid metabolites sharing a common structure (Carlstead et Brown, 2005; Lynch, Ziegler et Strier, 2002; Möstl et Palme, 2002; Möstl, Rettenbacher et Palme, 2005; Mateo, 2007; Young et Monfort, 2009). These metabolite antibodies typically yield higher concentrations of measured metabolites and display a higher reactivity with the fecal glucocorticoid metabolites than antibodies targeting native glucocorticoids and are therefore more likely to detect small fluctuations in fecal glucocorticoid metabolites (Morrow et al., 2002; Frigerio et al., 2004).

In this study, we validate and compare two fecal assays to monitor HPA axis activity in the eastern chipmunk (*Tamias striatus*) : one using an antibody raised against cortisol and another using an antibody raised against 5α-pregnane-3β, 11β, 21-triol-20-one. Cortisol is the main glucocorticoid secreted by all sciurid species studied to date, including the yellow-bellied marmot *Marmota flaviventris*, California ground squirrel *Spermophilus beecheyi*, golden-mantled ground squirrel *Spermophilus saturatus*, red squirrels *Tamiasciurus hudsonicus*, and yellow-pine chipmunks *Tamias amoenus* (Boonstra et McColl, 2000; Kenagy et Place, 2000; Place et Kenagy, 2000; Boonstra et al., 2001). First, we performed a radiometabolism study to analyze the time lag between cortisol secretion into the blood and its excretion in the feces as well as the relative importance of the routes of excretion (urine vs. feces). We measured the radioactivity as well as immunoreactivity of each fraction with a metabolite antibody recognizing steroids with a 5α-3β-11β-diol structure (Touma et al., 2003) as well as with a cortisol antibody validated in a diversity of species (Young et al., 2004). The metabolite antibody enables a reliable monitoring of the HPA axis in related species, such as Columbian ground squirrels *Spermophilus columbianus* (Bosson, Palme et Boonstra, 2009) and red squirrels *T. hudsonicus* (Dantzer et al., 2010). Second, we monitored baseline fecal cortisol metabolite levels of captive individuals over 2 d to determine the pattern of the circadian rhythm excretion. We also monitored fecal cortisol metabolite levels after stimulation of the adrenal by an ACTH injection to ensure that our method detects changes in blood cortisol levels. Finally, we compared both assays regarding their suitability to noninvasively measure adrenocortical activity in eastern chipmunks. While assays against the

parent hormone and the assays against the metabolites have successfully been applied to study glucocorticoid levels in free-ranging populations, few studies compare these two types of assays. Consequently, our study will expand our knowledge on the relative advantages of these two types of assays applied to wild populations.

2.3 Methods

2.3.1 Capture and housing of chipmunks

We livetrapped nine chipmunks (five males and four females) on the campus of the University of Toronto at Scarborough (43°47 N, 79°11 W, elevation = 116 m) in August 2010. Longworth traps were baited with sunflower seeds, apples, and carrots, opened at dawn, inspected every 90 min, and closed around 15 h. Females showing signs of lactation were immediately released at the point of capture. We housed chipmunks individually in plastic cages (47 cm x 26 cm x 20 cm) equipped with a wire bottom and a water bottle with a stainless steel nipple. Cages were mounted within a second same-sized tray, equipped with a fine metal mesh. This system enabled the feces and urine to fall through the cage bottom, but the feces were caught on the fine wire mesh while the urine passed through, thus preventing contamination of the feces by the urine. Animals sometimes urinated onto the feces, in which case the fecal pellets were discarded. Hence, we were able to collect the samples while minimizing disturbance of the chipmunks. Each chipmunk was provided with a plastic refuge, a piece of natural wood, and some cotton as bedding material. They had *ad lib.* access to rodent chow pellets, peanut butter, sunflower seeds, apples, carrots, and occasionally acorns. Chipmunks were kept at 20°C under natural photoperiod for the duration of the experiment. Except during collection of the samples, chipmunks were not exposed to human presence or noise. Chipmunks were left to acclimate for at least 5 d before beginning the trials. During that phase, feces were collected every 4 h between 0800 and 2200 hours to habituate them to the procedure (see Table 2.1 for the chronology of the tests and the sampling times). Upon completion of the experiment, we inspected and weighed the chipmunks.

Chipmunks did not lose weight or show any sign of deteriorating health related to the trials. However, we witnessed a decrease in activity during the last 3 d of the trials that paralleled the decrease in chipmunk aboveground activity frequently observed in nature during the late summer (Dunford, 1970; Elliott, 1978). All husbandry and manipulations were conducted under University of Toronto animal use protocol 20008380, issued in accordance with the Canadian Council on Animal Care guidelines.

Tableau 2.1 Chronology of the manipulations carried out on nine eastern chipmunks in August 2010.

Date	Procedure	Treatment	Collection time for samples
7th to 8th	Capture		
8th to 11th	Acclimation	No manipulation	Every 4h from 0800 to 2200.
12th to 15th	Radio-metabolism study	Injection of radiolabelled cortisol	Every 2h from 0800 to 2200.
16th	Baseline monitoring	No manipulation	Every 4h from 0800 to 2200.
17th to 18th	ACTH challenge	Injection of synthetic ACTH	Every 2h from 0800 to 2200.
19th	Baseline monitoring	No manipulation	Every 4h from 0800 to 2200.

2.3.2 Route and time course of cortisol excretion

To monitor the route and timing of cortisol excretion, we injected the nine chipmunks intraperitoneally with 1,110 kBq of radiolabeled cortisol (1,2,6,7-[^{3}H]-cortisol; Amersham Biosciences; specific activity p 1.55 TBq/mmol). Before injection, radiolabeled cortisol was dried down under air and reconstituted in 10 % ethanol and 90 % sterile physiological saline solution in order to inject a volume of 300 mL. Chipmunks were

injected between 0700 and 0800 hours, and urine and feces were then collected for 72 h (see Table 2.1 for sampling intervals). We collected all dry (i.e., uncontaminated) feces pellets with forceps and collected all urine using a 1-mL pipette. We rinsed the collecting trays with 2 mL of methanol to collect the remaining radioactivity and added these to the urine sample. Feces mixed with urine were also collected and analyzed separately. All samples were immediately put on ice and frozen at - 20 °C within 20 min of collection. Urine samples were dried down under air and reconstituted in 1 mL of methanol (80 %). We dried the fecal samples at 70 °C for 3 d (until they reached constant mass; dried radioactive samples ranged from 5 to 50 mg). We then crushed the feces using a plunger and extracted each whole sample by vortexing the pulverized feces with 1 mL of methanol (80 %). We quantified activity in the samples by adding 2.5 mL of scintillation fluid to 0.5 mL of urine or fecal extract and measured radioactivity with a scintillation counter with quench correction (Tri-Carb 2900TR, Boston, MA).

2.3.3 Characterization of fecal cortisol metabolites

Six fecal extracts with maximum radioactivity (three samples from males and three from females) were dried down under air and sent to University of Veterinary Medicine, Vienna, Austria. The radioactive cortisol metabolites in these samples were separated according to their polarity by reverse-phase high performance liquid chromatography (HPLC). We measured radioactivity and immunoreactivity in each fraction with the 5α-pregnane-3β, 11β, 21-triol-20-one EIA described below (Touma et al., 2003; Lepschy et al., 2007). In addition, we quantified the amount of immunoreactive cortisol present in the fecal samples by measuring the immunoreactivity of each fraction with a cortisol EIA. Additional details on the assay can be found elsehwhere (Palme et Möstl, 1997).

2.3.4 Monitoring baseline and stimulated cortisol secretion

To validate the ability of the EIAs to detect changes in cortisol secretion patterns, we injected animals with synthetic adrenocorticotropic hormone (4 IU/kg, ACTH; Synacthen Depot, CIBA). Fecal samples were collected to measure baseline levels of fecal

cortisol metabolites 24 h before and 48 h after ACTH injection. Animals were injected between 0700 and 0800 hours. After injection, we collected samples from 0800 to 2200 hours for 48 h (see table 2.1 for frequency of sampling).

2.3.5 Extraction and analysis of fecal cortisol metabolites

We dried down fecal samples at 70 °C for 72 h (samples typically reach constant mass after 48 h). Dried samples were then transferred to a 2-mL test tube and ground using a plunger. We extracted the fecal cortisol metabolite by vortexing 35 $\pm$ 5 mg of ground feces in 1 mL of methanol 80 % for 30 min at 1500 rpm and centrifuging (2500 g; 20 min). We then analyzed the supernatant with a 5α-pregnane-3β, 11β, 21-triol-20-one EIA (Touma et al., 2003). Details about cross-reactivity of the antibody and the assay procedure can be found in Touma et al. (2003). Mean intra- and interassay coefficients of variation of two pool samples were 11.5 % and 16.5 %, respectively. To compare assays, we also analyzed the samples collected during baseline monitoring and after ACTH injection with a cortisol EIA. The cortisol EIA used a polyclonal cortisol antibody (R4866; C. J. Munro, University of California, Davis) with a horseradish peroxidase ligand (see Munro and Lasley 1988 for details on cross-reactivity; see Young et al., 2004 for details on EIA protocol). Mean intra- and interassay coefficients of variation were 9.7 % and 9.9 %, respectively.

2.3.6 Statistical analysis

We tested for sex differences in time to peak excretion in feces and urine, percentage of injected radioactivity recovered, and the proportion of radioactivity excreted in the feces, using linear models. Fecal cortisol metabolite levels measured with the 5α-pregnane-3β, 11β, 21-triol-20-one and the cortisol assays were analyzed in a two-step manner. First, to explore the factor affecting cortisol baseline levels, we used a linear mixed model including hour of sample collection (categorical variable), sex, and their interaction as fixed effects. Individual identity was fitted as a random effect to account for repeated measurements of the same individuals over time, also known as pseudoreplication (Craw-

ley, 2007). A log-likelihood ratio test comparing a model including the random effect of interest and a model without it while holding the fixed effect structure constant was used to test for the significance of the random effect (Pinheiro et Bates, 2000). For the individual random effect, we also report the repeatability (r), defined as the variance associated with the effect divided by the sum of this variance and the residual variance from the model (Pinheiro et Bates, 2000). High r values are indicative of substantial, consistent differences among individuals. Second, to evaluate how ACTH injection affected fecal cortisol metabolite levels compared to baseline levels, we analyzed fecal cortisol metabolite levels in all collected samples (i.e., during baseline sampling and after ACTH injection) within one linear mixed model. This second model initially included hour of sample collection, time since ACTH injection, type of sampling (baseline monitoring or ACTH injection), sex, and their interactions as fixed explanatory variables. Again, we added chipmunk identity as a random effect to account for pseudoreplication and tested its significance using a log-likelihood ratio test as described above. All models were simplified by stepwise deletion of nonsignificant terms (Crawley, 2007). To compare the results obtained with both assays, we computed Pearson's product-moment correlation coefficient for all samples analyzed. We also compared the relative increase in fecal cortisol metabolite level detected by each assay after ACTH injection using a linear model. The response variable was the increase in fecal cortisol metabolite level of each individual expressed as the percentage of baseline level at the same time of day (the percentage was then log transformed). The explanatory variables were number of hours since ACTH injection, assay used (5α-pregnane-3β, 11β, 21-triol-20-one or cortisol assay), sex of individuals, and their interactions. All fecal cortisol metabolite levels are expressed as log-transformed values (units are ng/g dry feces). Statistical analyses were performed using R 2.10.1 (R Development Core Team, 2011). Means are reported with $\pm$ SEM.

2.4 Results

2.4.1 Route and time course of ^{3}H-cortisol excretion

During the 72 h following injection of radiolabelled cortisol, we collected 151 and 180 urine and fecal samples respectively. We recovered 48.33 ± 0.13 % of the 1110 kBq injected in each subject (range = 29.00 - 75.17). A total of 83.10 ± 6.67 % and 16.21 ± 6.86 % of the radioactivity recovered was found in the urine and the feces, respectively. The rest (0.69 ± 0.90 %) was found in the feces contaminated with urine. There was no difference in the percentage of radioactivity recovered between sexes (effect = 12.85 ± 8.5; t_7 = 1.51; p = 0.17) nor in the percentage of radiolabelled cortisol excreted via the feces (effect = -4.29 ± 4.65; t_7 =-0.92; p = 0.39). Median peak excretion of radioactivity in the urine occurred at 4 h after injection (range = 2 - 4, mean = 3.11 ± 1.05 h) and in feces 8 h after injection (range = 8 - 12, mean = 9.33 ± 1.73; see Fig. 2.1). Time to peak excretion in urine (t_7 = 0.27; p = 0.80) or in feces (t_7 = -1.04; p = 0.33) was not affected by sex.

2.4.2 Characterization of fecal cortisol metabolites

HPLC revealed that cortisol was almost completely metabolized in both females and males (see Fig. 2.2). The most prominent radioactivity peaks eluted between fractions 35 and 50 for both sexes. Females produced more metabolites with higher polarity (fractions 10 to 20). Although there were differences between sexes, both EIAs were able to recognize several radioactive peaks.

2.4.3 Monitoring baseline and stimulated (ACTH injection) cortisol secretion

Baseline fecal cortisol metabolite levels (N = 72 samples) analyzed with the 5α-pregnane-3β, 11β, 21-triol-20-one EIA ranged from 1720 to 15000 ng/g of dry feces (median = 5390 ng/g) and did not vary with sex at the 0800 sampling (effect = -0.19 ± 0.19, t_{57} = -0.96, p = 0.37). However, males tended to show higher fecal cortisol metabolites levels during

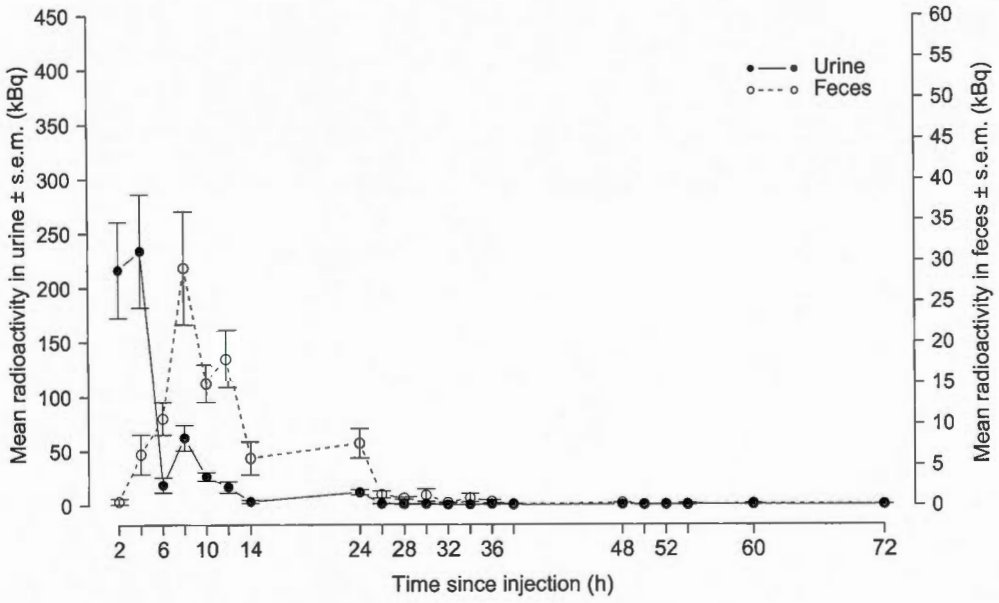

Figure 2.1 Radioactivity (mean ± s.e.m.) excreted in urine (closed dots, solid line) and feces (open dots, dashed line) in nine eastern chipmunks following an injection of radio-labelled cortisol.

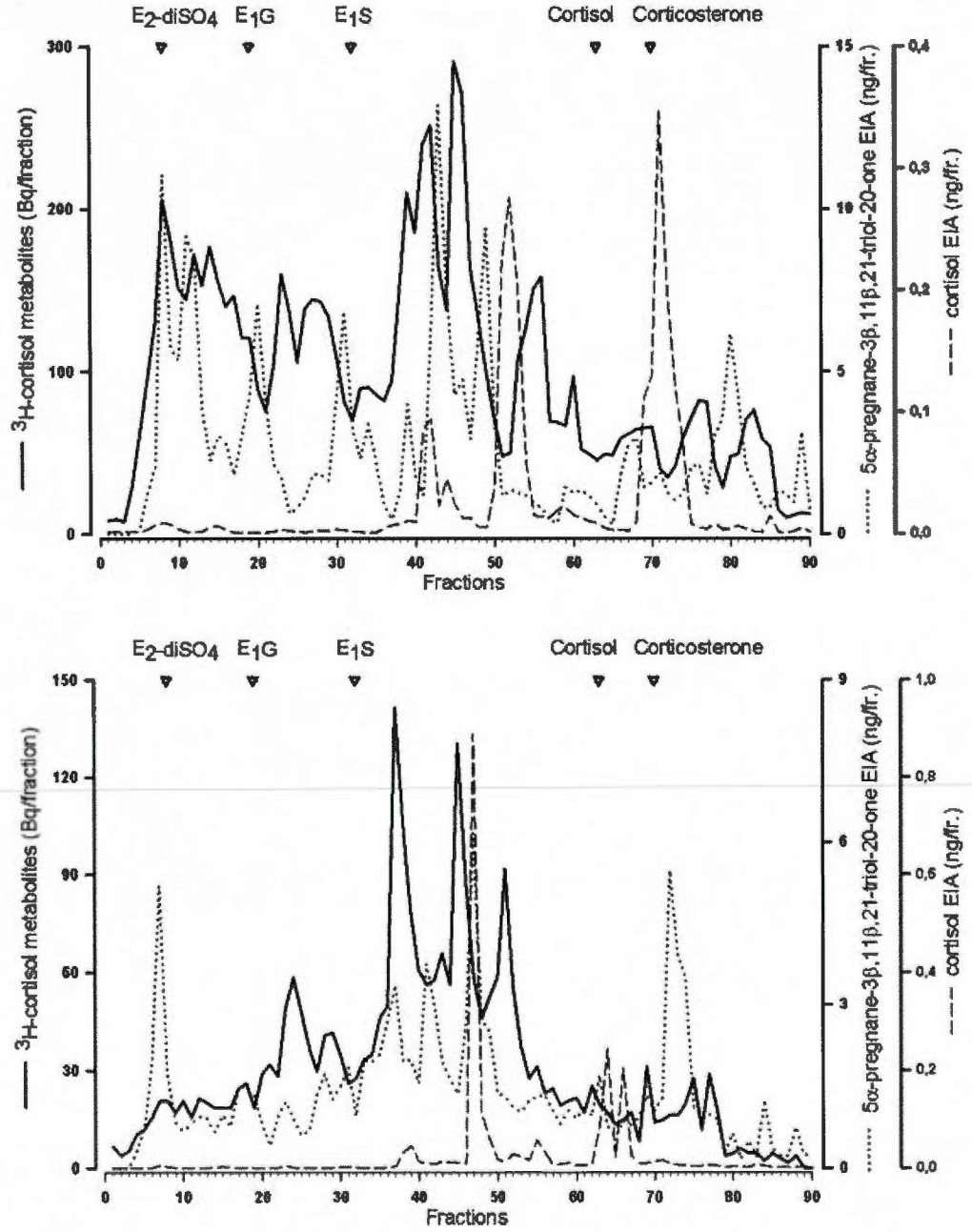

Figure 2.2 Radioactivity (solid line) and immunoreactivity determined by a cortisol (dashed line) and a 5α-pregnane-3β, 11β, 21-triol-20-one (dotted line) enzyme immunoassays of female (upper panel) and male (lower panel) eastern chipmunk fecal extracts fractionated by HPLC. Open triangles mark the approximate elution time of estradiol disulphate (E2-dSO_4), estrone glucuronide (E1G), estrone sulfate (E1S), cortisol and corticosterone standards.

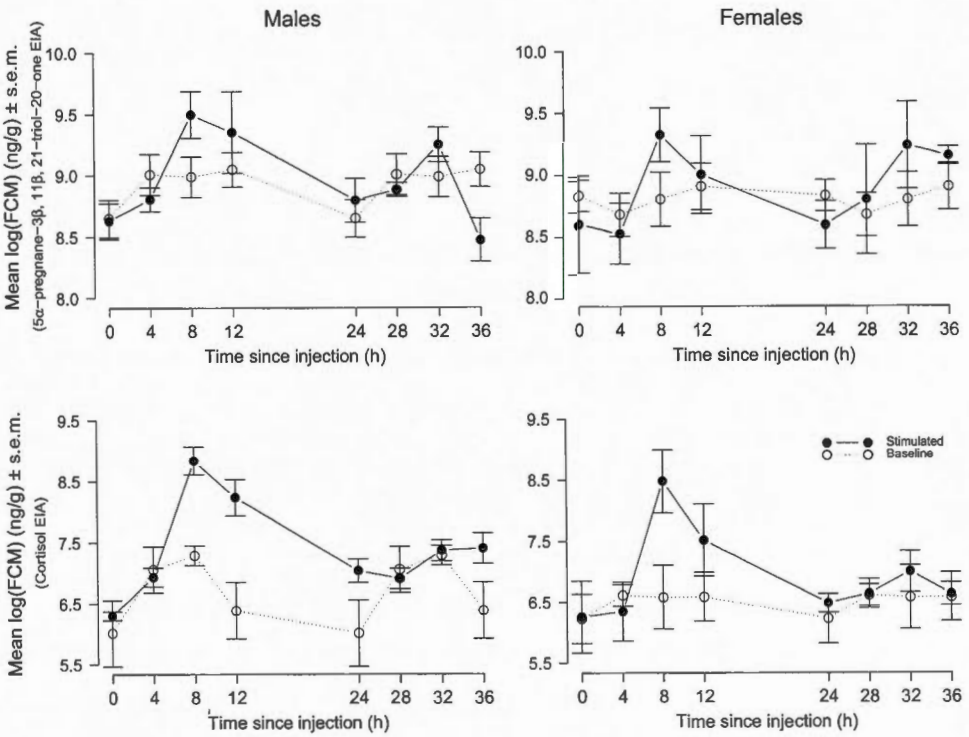

Figure 2.3 Concentrations of fecal cortisol metabolites (FCM) in male (N = 5) and female (N = 4) eastern chipmunks during baseline (open dots, dotted line) and following an ACTH injection (closed dots, solid line). FCM levels were measured using a 5α-pregnane-3β, 11β, 21-triol-20-one (upper panels) and a cortisol (lower panels) enzyme immunoassay.

the rest of the day (all effects <0.34, all p <0.04 ; see upper section of Fig. 2.3). Animals also displayed consistent individual differences regarding their fecal cortisol metabolite level (r = 51.22 %, log-likelihood ratio = 25.67, p <0.001). The ACTH injection (N = 72 samples) affected fecal cortisol metabolite levels $\sim$ 8 h after the injection (effect = 0.51 $\pm$ 0.15, t_{116} = 3.38, p = 0.001 ; see upper section of Fig. 2.3). Again, we detected important individual differences in the mean fecal cortisol metabolite levels (r = 46.72 %, log-likelihood ratio = 53.16, p <0.001).

Analysis of the baseline fecal cortisol metabolite levels with the cortisol EIA ranged from 133 to 2942 ng/g of dry feces (median = 700 ng/g) and revealed that males displayed

higher fecal cortisol metabolite levels than females at 1200 (effect = 0.79 ± 0.30, t_{51} = 2.59, p = 0.012) and 1600 h (effect = 1.01 ± 0.31, t_{51} = -0.18, p = 0.002; see lower panel of Fig. 2.3). The cortisol EIA also revealed important individual variation (r = 72.20 %, likelihood-ratio = 50.04, p <0.001). This EIA also detected an effect of ACTH injection on fecal cortisol metabolite levels with effects at 1600 h (effect = 1.92 ± 0.46, t_{51} = 4.14, p = 0.001) and 2000 h (effect = 1.67 ± 0.50, t_{51} = 3.32, p = 0.001). This EIA detected important individual variation in the mean fecal cortisol metabolite levels (r = 40.26 %, log-likelihood ratio = 41.05, p <0.001). Concentrations of fecal cortisol metabolites measured with both EIAs were significantly correlated (r = 0.45, N = 138, p <0.001).

Analysis of the relative increase in fecal cortisol metabolite detected by each assay (5α-pregnane-3β, 11β, 21-triol-20-one assay : median = 101 %, range = 36 - 556; cortisol assay : median = 132 %, range = 16 - 1385) showed that the cortisol EIA measured a higher increase relative to the baseline samples 8 and 12 h following ACTH injection compared with the 5α-pregnane-3β, 11β, 21-triol-20-one EIA (8 h sampling : effect = 1.28 ± 0.39, t_{106} = 3.25, p = 0.001; 12 h sampling : effect = 1.11 ± 0.39, t_{106} = 2.81, p = 0.005; one outlier was excluded from the analysis; see Fig. 2.4). Relative increase in measured FCM did not vary between both EIAs during all the remaining sampling times following ACTH injection (all effects <0.50, all p >0.20). We did not find any statistically significant difference between the sexes in the relative increase in fecal cortisol metabolite with either assay (all terms rejected with p >0.18).

2.5 Discussion

The objective of this study was to assess glucocorticoid levels of eastern chipmunks non-invasively by validating the measurement of fecal cortisol metabolites using two different EIAs. The first used a cortisol antibody, while the second, using a 5α-pregnane-3β, 11β, 21-triol-20-one antibody, targeted a group of its metabolites. Our key conclusion is that both antibodies appear to accurately represent fecal metabolite levels, but that the cortisol EIA is more sensitive. The radio-metabolism experiment showed that only about

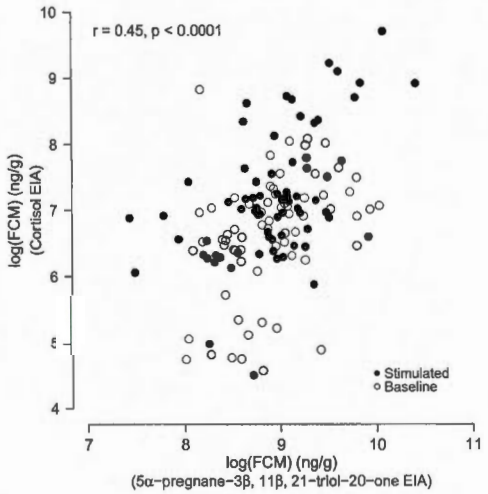

Figure 2.4 Fecal cortisol metabolites following an ACTH injection for male (N=4) and female (N = 4) eastern chipmunks. Levels are expressed as a percentage of baseline levels (mean ± s.e.m.) measured at the same time of day. Fecal cortisol metabolites were measured using a 5α-pregnane-3β, 11β, 21-triol-20-one (open dots) and a cortisol (closed dots) enzyme immunoassays. One outlier male was excluded from the analysis.

16 % of the metabolites are excreted in feces and that the time lag between secretion of cortisol into the blood and the appearance of its metabolites in the feces was about 8 h. We found that males displayed a circadian rhythm with a peak concentration at 1600, whereas females did not. Following an ACTH stimulation test, we found that both assays detected an increase in cortisol metabolites, which was expressed more strongly in the cortisol EIA. Finally, both assays detected ample individual variation, showing that animals consistently differed from each other regarding their fecal cortisol metabolite levels. We discuss each of these points below.

Our radio-metabolism study showed that chipmunks excreted most of the injected cortisol in urine (83 %) within the first 4 hours following injection of radiolabelled cortisol. In contrast, we detected a peak in excretion in the feces 8 hours after the injection. Blood cortisol is metabolized in the liver and excreted into the gut via the bile (Möstl et al. 2005). The time lag between radiolabelled cortisol injection and its peak excretion in the feces can be attributed mostly to the time needed for the intestinal transit (Palme et al., 2005). Similar lag-times have been described in the Columbian ground squirrels, $\sim$ 7 h, (Bosson, Palme et Boonstra, 2009). However, chipmunks exhibit a shorter time lag than red squirrels, 10.9 h, (Dantzer et al., 2010). We were surprised to recover only $\sim$ 50 % of the injected radioactivity, given that we collected all feces and urine puddles under as well as within the cage. This loss could be attributed to absorption of the radioactivity from the urine by the plastic of the cages, as all other accessories in the cage had been removed for the duration of the radiometabolism study. If this is the case, our results would tend to overestimate the proportion of radiolabelled cortisol excreted via the feces. A portion of this loss may also be attributed to radioactivity loss during metabolism. Indeed, some of the tritium incorporated into the cortisol molecules may be exchanged for non-radioactive hydrogen during metabolism. Using a more stable radioisotope (i.e. ^{14}C) to label the cortisol would have avoided this bias. However, 14C-labelled glucocorticoids are very expensive, as they require complex synthesis protocols (Möstl et al. 2005). In accordance with previous radio-metabolism studies carried out in other species, our HPLC analyzes of fecal samples show that ^{3}H-cortisol was

extensively metabolized. Native cortisol was only found in small amounts, if at all, in the feces (Touma et al., 2003; Palme et al., 2005; Lepschy et al., 2007). Nevertheless a cortisol EIA was able to react with some of the radioactive metabolites. Due to the cross-reactions of the used antibody (antigen : cortisol-3-carboxymethyloxime linked to bovine serum albumin) these metabolites should have a 11α,17β,21-triol-20-one structure (e.g. tetrahydrocortisol). The 5α-pregnane-3β, 11β, 21-triol-20-one assay detected more metabolites and had higher peak levels. Proper identification of the metabolites would require substantial effort (including mass-spectrometry, and steroid standards no longer available), which are beyond the aim of this study. In addition, our results show once again that expressed differences in excreted fecal glucocorticoid metabolites are present even in closely related species (Bosson, Palme et Boonstra, 2009; Dantzer et al., 2010).

Both assays detected a significant increase in immunoreactivity following stimulation of the adrenals (with an injection of ACTH). Thus, our study validates both EIAs. Such a physiological validation is critical, as it ensures that an assay is able to detect changes in endogenous cortisol production by the adrenal glands (Möstl, Rettenbacher et Palme, 2005; Palme et al., 2005; Touma et Palme, 2005). Moreover, the time course of fecal cortisol metabolite levels after an ACTH injection was in agreement with the time course of cortisol excretion evidenced by the radio-metabolism study, with a peak excretion occurring in the feces around 8 h after adrenal stimulation. Thus, both assays are suited to monitor cortisol production in the eastern chipmunk. We nevertheless detected important differences regarding the reactivity of each assay to an increase in blood cortisol. Indeed, whereas the 5α-pregnane-3β, 11β, 21-triol-20-one assay detected a mean relative increase of $\sim$ 100 % following ACTH injection, the cortisol EIA displayed a larger reactivity (relative increase of $\sim$ 500 % when the sexes are pooled). An increased reactivity is traditionally thought to result into a greater sensitivity (Morrow et al., 2002; Möstl, Rettenbacher et Palme, 2005). Therefore the cortisol assay validated in this study should detect smaller fluctuations in fecal cortisol metabolite levels (Frigerio et al., 2004). These findings contrast with previous studies reporting a higher reactivity

of metabolite assays compared to cortisol/corticosterone assays (Morrow et al., 2002; Frigerio et al., 2004). However, others also reported a cortisol EIA being better suited in some primate species (Heistermann, Palme et Ganswindt, 2006). In addition, an assay targeting another group of fecal metabolites (11-oxoetiocholanolone EIA) turned out to be better suited than the 5α-pregnane-3β, 11β, 21-triol-20-one EIA in other squirrel species (Strauss et al., 2007; Sheriff et al., 2011b). Thus our results once more underline the importance of validating each assay in a given species (Palme, 2005; Touma et Palme, 2005; Sheriff, Krebs et Boonstra, 2011).

The assays we validated indicated significant gender differences in circadian patterns of baseline fecal cortisol metabolite levels. Circadian rhythms in fecal cortisol metabolite levels have been detected in Columbian ground squirrels (Bosson, Palme et Boonstra, 2009), but not in red squirrels (Dantzer et al., 2010). While male chipmunks displayed an increase in cortisol metabolite levels that peaked around 1600 h each day, females did not. Circadian patterns in cortisol metabolite levels should reflect circadian fluctuation in blood levels. Animals typically display a peak in cortisol secretion into the blood at the beginning of their active phase, which is thought to enable increased locomotion and foraging behaviour (Reeder et Kramer, 2005). Accounting for the 8 h lag time, our results suggest that the peak cortisol secretion into the blood is located around dawn. The circadian variation in cortisol metabolites we observed in this study is thus in accordance with what we would predict. Studies reporting circadian cortisol variation in small mammals typically report more pronounced variation in females than in males (Touma, Palme et Sachser, 2004; Cavigelli et al., 2005; Lepschy et al., 2007). In our case, we did not detect circadian variation in females. Sex differences over the circadian rhythm of fecal cortisol metabolites could arise from differences in activity patterns between sexes, or because of differences in metabolite composition between males and females. As suggested by Touma et al. (2003), activity pattern affects the timing and frequency of fecal excretion, and might thus modify circadian fecal cortisol metabolite patterns. In our case, however, we did not detect any difference between males and females in the time course of excretion (see 'Results'), suggesting that the differences we

witnessed between males and females cannot be explained by sex-differences in activity patterns. Males and females have already been shown to excrete different glucocorticoid metabolites in other rodent species (Touma et al., 2003; Lepschy et al., 2007). In our study, females produced more polar metabolites, as evidenced by the increased radioactivity eluted in the fractions 10 to 20. In addition, the assays seem to recognize different metabolites in both sexes.

Finally, both assays detected ample individual variation. Such individual variation was absent in the radio-metabolism study, and thus we cannot invoke individual differences in excretion patterns to explain individual variation detected by the assays. Consistent differences in fecal glucocorticoid levels among individuals are repeatedly reported in the literature (Touma et Palme, 2005; Bosson, Palme et Boonstra, 2009). In our study, individuals were kept in a standardized environment, and such differences could reflect individual differences related to components of the hypothalamic-pituitary-adrenal axis (for example, some individuals could display an increased adrenal baseline activity). When encountered in the field, individual differences in fecal cortisol metabolite levels might also originate from reproductive or social status differences among animals and from environmental characteristics such as temperature or predation risk (Sapolsky et Ray, 1989; Frigerio et al., 2004; Travers et al., 2010). Cortisol levels can also be associated with resource allocation decisions of animals relative to reproductive or survival functions (Ricklefs et Wikelski, 2002). Individual differences in cortisol levels are related to continuous behavioural variation over the stress response, as well as individual variation in reproductive tactics or life history that characterize some species (Overli et al., 2007; Lancaster et al., 2008; Koolhaas et al., 2010; Réale et al., 2010a). Disentangling the effects of these factors on cortisol levels and explaining consistent individual variation in cortisol production requires the monitoring of cortisol levels over extended periods of time within and among individuals and in natural environments. The assays we validated in this study will enable such studies in the chipmunk, and will thus contribute to understanding the roles glucocorticoids play in the expression of adaptive individual variation (Wingfield, Visser et Williams, 2008).

2.6 Conclusion

In conclusion, our study investigated the timing and route of excretion of cortisol metabolites and validated two enzyme immunoassays to monitor adrenocortical activity in the eastern chipmunk. Both assays provide a valid method of measuring cortisol levels secreted by individuals around 8 h before sampling. The cortisol assay displayed higher reactivity. These immunoassays will enable longitudinal studies of the cortisol levels in free-ranging individuals, providing a valuable tool to study the physiological bases of individual variation in eastern chipmunks.

2.7 Acknowledgements

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CHAPITRE III

PERSONALITY DIFFERENCES ARE RELATED TO LONG TERM STRESS REACTIVITY IN WILD EASTERN CHIPMUNKS (*TAMIAS STRIATUS*)

Pierre-Olivier Montiglio, Dany Garant, Fanie Pelletier, & Denis Réale

3.1 Abstract

Consistent individual behavioural differences in exploration, docility and boldness are often correlated and are associated with differences in short-term neurophysiological responses to environmental perturbations in many animal species. These physiological mechanisms are much less studied over longer periods in wild populations. Here we report the relationships among exploration, docility measured in open-field tests and trappability, taken as an index of boldness, in a wild population of eastern chipmunks and investigate whether behavioural differences among individuals are associated with differences in autonomic nervous system reactivity. We also assess the cortisol level of individuals over several months to investigate whether chipmunks with different exploration levels display different mean cortisol levels or differences in their cortisol variability. Open-field tests showed consistent individual differences in exploration patterns (ranging from fast to slow). Faster explorers were less docile when handled and were trapped more often (males) or farther from their burrows (females) than slower explorers. Fast explorers also showed a higher sympathetic activity under restraint but more stable cortisol levels over the course of the active season, suggesting a lower hypothalamo-pituitary-adrenal reactivity. Our results show that chipmunks dis-

play individual behavioural variation and that these differences may have physiological implications over long periods in natural settings. Future studies should investigate the fitness consequences of such behavioural/physiological differences.

3.2 Introduction

Animals vary consistently in their behavioural response to environmental perturbations (Wilson, 1998; Koolhaas et al., 1999; Sih, Bell et Johnson, 2004; Réale et al., 2007). Behavioural traits are also often correlated so that, for example, individuals that are more active (i.e. faster explorers) are also more aggressive, less docile and bolder (Koolhaas et al., 2010; Réale et al., 2010b). Faster explorers are also expected to use more unprotected, open areas of their environment compared to slower explorers (Koolhaas et al., 1999). For example, red squirrels, *Tamiasciurus hudsonicus*, that are more active in a novel environment are also bolder (based on their propensity to enter traps), less docile when handled by humans and more often captured on the territories of conspecifics (Boon, Réale et Boutin, 2007; Boon, Boutin et Réale, 2008). A key challenge is to understand the causes of such individual differences (Koolhaas et al., 1999; Sih, Bell et Johnson, 2004; Réale et al., 2007).

Individual differences in boldness, exploration and aggressiveness are related to differences in the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, regulating how individuals react to environmental perturbations or stress (Koolhaas et al., 1999; Boonstra, 2005; Reeder et Kramer, 2005). Laboratory studies conducted in standardized environments have shown that faster explorers display a higher reactivity of the sympathetic autonomic system than slower explorers (Carere et van Oers, 2004; Fucikova et al., 2009), which is associated with increased heart rate and heart rate variability (Visser et al., 2002). Faster explorers also have a lower hypothalamic-pituitary-adrenal reactivity, leading to reduced cortisol secretion during the minutes/hours following the challenge (Carere et al., 2005; Overli et al., 2007). However, while such physiological mechanisms are well studied over short timescales and in relation to standardized challenges, their importance is much less investigated over the long term (months/the

lifetime of individuals) and in uncontrolled natural settings. As a result, it is still unclear whether differences expressed in standardized tests are still detectable and relevant in a natural environment. Furthermore, information on the relationships between short-term and long-term physiological responses to stress is still scarce (Carlstead et Brown, 2005). Koolhaas et al. (1999) suggested that reactive individuals should display a higher baseline cortisol level but also a higher variability in their cortisol production over longer timescales, a measure seldom considered in natural settings (but see Carlstead et Brown, 2005 for an example in captive animals). Others have hypothesized that short-term increases in cortisol responses may not affect overall cortisol levels and may be negligible over the long term (Goymann, 2005). Further tests conducted under natural conditions are therefore critically needed to decipher the relationships between short-term and long-term responses to stress.

In this study, we tested the hypothesis that exploration, docility and boldness share the same physiological bases in a wild population of eastern chipmunks (Koolhaas et al., 2010; Réale et al., 2010b). More specifically, we quantified activity level in a novel environment, docility during handling by humans (i.e. a bag test), the propensity to enter traps (i.e. trappability) and the propensity to use a wider space area, often taken as an index of boldness (Wilson, 1998; Réale et al., 2000) among individuals of this population. We took advantage of an extensive survey on a free-ranging population in southern Québec, where all individuals on a trapping grid were marked, observed and tested for exploration between 2005 and 2009 (Landry-Cuerrier et al., 2008; Montiglio et al., 2010). Individuals in this population varied in their level of activity in a novel environment (during open-field tests), from 'slow' to 'fast' explorers (Montiglio et al., 2010). Based on the hypothesis that bold, less docile and fast-exploring individuals should display a lower cortisol reactivity and higher autonomic system reactivity (Koolhaas et al., 2010), we predicted a positive relationship between exploration speed in the open field and boldness (trappability), and a negative relationship between exploration speed and docility during bag tests (Koolhaas et al., 2010; Réale et al., 2010b). We also combined behavioural and physiological measurements to investigate

the relationship between exploration and the sympathetic autonomic system reactivity by monitoring heart rate during a restraint test. We predicted that faster explorers would display a greater increase in heart rate when restrained. Finally, we assessed the relationship between exploration and hypothalamic-pituitary-adrenal activity over an entire season (5 months), through the analysis of faecal cortisol metabolite levels (Palme, 2005; Montiglio et al., 2012b). This measure represents an integrated measure of the animal's hypothalamo-pituitary-adrenal (HPA) activity over a few hours (Touma et Palme, 2005). If individual differences in short-term stress response affect the long-term levels of cortisol, then we predicted that faster explorers would express lower and less variable faecal cortisol metabolite levels over the season. Alternatively, if individual differences in short-term stress response are independent of the long-term cortisol level, then we expected no relationship between exploration speed and faecal cortisol levels.

3.3 Methods

Model Species and Study Site

Eastern chipmunks are solitary ground-dwelling sciurids found in eastern North America (Snyder, 1982). They feed mostly on seeds, nuts and acorns from masting trees (Landry-Cuerrier et al., 2008). In the northern part of its range, the eastern chipmunk is active from late March to October, and spends the winter in its burrow on food stocks hoarded during the summer (Elliott, 1978). The eastern chipmunk has a promiscuous mating system (Bergeron et al., 2011b), with intense scramble competition among males (Elliott, 1978).

3.3.1 Trapping of Chipmunks and Bag Tests

The study site was a 500 x 500 m grid of deciduous forest located in southern Quebec (45°05'N, 72°25'W) where American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*) dominated. We trapped chipmunks in Longworth traps baited with peanut butter each year during the active season from 2005 to 2009. Upon each capture and

before any manipulation, we transferred each chipmunk from the trap to a mesh bag and counted the number of seconds it spent immobile during 1 min as a measure of its docility (Martin et Réale, 2008). We identified chipmunks using two metal eartags (National Band and Tag Co., Newport, KY, U.S.A.) and Trovan passive integrated (PIT) tags (EIDAP, Inc., Sherwood Park, Alberta, Canada). Individuals were tested in an open-field apparatus twice during their lifetime and released at the point of capture (see below).

3.3.2 Open-field Tests

Chipmunks subjected to the open-field test were immediately transferred from the trap to a handling bag. We identified the chipmunk without any manipulation with a hand-held PIT tag reader and transferred it to a small chamber connected to a white plastic arena (80 x 40 x 40 cm or 80 x 80 x 40 cm; see below) with lines drawn on the floor. We introduced the animal into the arena by lifting a small door connecting the chamber and the arena. We videotaped the behaviour of the chipmunk from above for 90 s. We coded its behaviour in the open field using The Observer 5.0 software (Noldus Information Technology, Wageningen, The Netherlands). For each test, we measured activity as the number of lines crossed during three 30 s intervals. Coding the behaviour of chipmunks on three successive intervals during each test enabled us to analyse temporal patterns of behaviour within tests (Montiglio et al., 2010). We coded a subsample of open-field tests (N = 2 tests on 39 individuals) using a detailed ethogram derived from the one used by Martin & Réale (2008). Based on these results, for the other tests, we coded the total number of line crosses ('ambulation'), the time spent scanning ('scan'), as well as the proportion of time spent in the centre of the arena ('centrality'). In both 2006 and 2007, we used an arena (80 x 40 x 40 cm) equipped with holes at the bottom (i.e. a modified hole-board test), described in Martin & Réale (2008). As the holes on the bottom had no detectable effect on the chipmunks behaviour (Montiglio et al., 2010), we used a bigger arena (80 x 80 x 40 cm) without holes for subsequent tests. Preliminary analyses showed that changing the arena size did not affect chipmunks' behaviour, but

we nevertheless included the size of the arena in all the statistical models related to the open-field tests (see Statistical Analyses).

3.3.3 Trappability

We used the trapping data set to estimate individuals' propensity to enter in a trap. To do so, we combined all the traps that were opened on a particular day (referred to as 'trap-days'). We assigned each individual a value of 1 (captured) or 0 (not captured) for each trap-day. We restricted our analyses to available trap-days in 2008 and 2009 and to adult chipmunks only. This approach enabled us to model the probability of capturing an individual as a function of the distance from the centre of its trapping range (see below) while correcting for potentially confounding variables. We estimated the centre of an individual's trapping range by computing the centre of the minimum convex polygon (MCP) including all the locations where the individual was captured in a given year (Calenge, 2006). Because we used available trap-days as the model's observation unit, this method excluded statistical artefacts caused by border effects, inherently associated with analyses of trapping data on a finite-size grid.

3.3.4 Hormone Assays

We collected faecal samples from the traps and the manipulation bags and kept them on ice. We transferred all samples to a - 20°C freezer within 8 h of sampling. Samples were stored for 2 weeks at - 20°C, then stored at - 80°C until analysis. We dried the samples at 70°C and pulverized them using a plunger. We extracted faecal cortisol metabolites by vortexing approximately 35 mg of samples in 1 ml of methanol (80 %) for 20 min (15000 revolutions per minute). We centrifuged the liquid and collected the supernatant for an enzyme immunoassay using a cortisol antibody with a horseradish peroxidase ligand (R4866, Coralie Munro, University of California, Riverside, CA, U.S.A., see Young et al., 2004). This assay has been previously validated using ACTH challenge tests, as well as by monitoring circadian variation in faecal cortisol levels (Montiglio et al., 2012b). Samples were analysed in duplicates, and we reanalysed all samples with coefficients of

variation (CV) >20 %. Intra- and interassay CVs were 8.4 % and 9.9 %, respectively.

3.3.5 Heart Rate Measurements

We measured heart rate during the first capture of individuals born in 2008 using a digital voice recorder placed on the thorax of the individuals. Individuals were gently taken out of the traps and constrained in the manipulation bag to avoid movements. Heart rate measurements were then taken less than 30 s following transfer of animals to the bag and lasted 1 min. To estimate heart rate, we measured the time (in ms) between two heart beats at 12 randomly chosen points from each audio recording using Audacity software (v. 1.3.13-beta, www.audacity.sourceforge.net).

3.3.6 Ethical Note

Open-field tests were not performed on juveniles weighting less than 50 g or on pregnant females. Some individuals were consistently recaptured for up to 4 years on the grid, and a survival analysis showed no significant effect of trapping on survival (Bergeron et al., 2011a). Animal captures, manipulations and tests were conducted following the guidelines of the Canadian Council on Animal Care through Université du Québec à Montréal (permits CIPA 0603-462-0607 and 0507-613-0509).

3.3.7 Statistical Analyses

We analysed the behaviour of chipmunks during the open-field tests using a principal components analysis (Legendre et Legendre, 2011). Prior to their inclusion in the analysis, behavioural variables were inspected for normality, centred and scaled. The proportion of time spent standing on hindlegs, and the proportion of time spent immobile were transformed as presence/absence data. The number of line crosses and the number of jumps were square-root transformed. We named each principal component axis based on the behaviour variable that was associated with the highest positive eigenvalue (i.e. ambulation, the number of line crosses during the test; scan, the time spent scanning

the environment; and centrality, the proportion of the time spent in the centre of the arena).

We analysed behaviours with the highest loading on the principal components using a linear mixed model (Pinheiro et Bates, 2000). For each model, we first included all the relevant interactions and main effects, and simplified it by rejecting all nonsignificant terms (P >0.05) one at a time (Crawley, 2007). The final models were used to compute individual coefficients describing the mean ambulation, scan or centrality levels of each individual. These coefficients are the best linear unbiased predictors (BLUPs, (Pinheiro et Bates, 2000); also see below and Supplementary Material). We quantified the consistency of individual behavioural differences by estimating the repeatability (r) as $100*(V_i/(V_i+V_r))$, where V_i is the variance associated with the individual random effect, and V_r is the residual variance of the model. Repeatability can be interpreted as the percentage of the total phenotypic variance explained by between-individual variance (Lessels et Boag, 1987). For each behaviour, we tested whether repeatability estimates were significantly different from 0 % using a one degree of freedom likelihood ratio test (LRT) that compared the likelihood of a model including the individual random effect to a new model without it (Pinheiro et Bates, 2000; Montiglio et al., 2010).

We used a similar approach to analyse the number of seconds spent active by a chipmunk during 1 min in the handling bag (log-transformed to satisfy normality). The linear mixed model initially included day of the test (centred on the year's median), year, sex and age class of the individual, the number of times the individuals had been captured before the test during the current year, and their two-way interactions as fixed effects. To determine whether the individual behaviour measured during the open-field test was related to the behaviour of the chipmunk while in the bag, we included individual BLUP values of ambulation, scan and centrality in the model (see above) and tested for all the two-way interactions between these values and the other fixed effects. We also included individual identity as a random effect to account for the fact that individuals were measured more than once (pseudoreplication). We then simplified the model and estimated the repeatability as described previously.

We analysed the probability of capturing an individual in the traps using a generalized linear mixed model (binomial family) with the package lme4, (Bates, Maechler et Bolker, 2011). Because the probability of capturing a given individual is likely to decrease with increasing distance from its home range, we included the distance between the trap location and centre of each chipmunk's trapping range in the model. We also included the year as well as the sex of the individuals in the initial model. BLUPs of ambulation, scan and centrality were included as fixed effects. To account for day-to-day variation in above-ground activity levels, we fitted the day of the test (categorical variable) as a random effect. We also fitted individual identity as a random effect to account for pseudoreplication. Preliminary analyses indicated sex differences in trappability (see Results), so we analysed males and females in separate models. We estimated the significance of random effects using a likelihood ratio test as described above.

We analysed faecal cortisol metabolite levels in samples with a linear mixed model. Date and time of sampling, sex and their interactions were included as fixed effects. To determine whether individual behavioural differences during open-field tests were related to differences in individuals' mean cortisol levels, we also included individual BLUPs of ambulation, scan and centrality, and tested for all two-way interactions between these and the other fixed effects. We also fitted chipmunk identity as a random effect to account for pseudoreplication. We simplified the model as described above until all remaining terms were significant. The faecal cortisol metabolite concentration was transformed using the function $Y = X^{0.2}$ to satisfy normality. We also investigated whether individual behaviour was associated with variability in cortisol level by computing the CV of faecal cortisol levels for each individual. Coefficients of variation were analysed with a linear model using number of samples available and mean faecal cortisol level of each individual. We also included individual BLUPs of activity, scan and centrality. The model was simplified as above until all remaining terms were significant. We investigated the relationship between the number of heart beats/s and individual behaviour in the open field using a linear model, where the response variable was the estimated number of heart beats/s and the explanatory variables were sex, ambulation, scan and central-

ity. We then simplified the model by stepwise deletion of nonsignificant terms until all remaining terms were significant.

3.4 Results

3.4.1 Open-field tests

The principal components analysis yielded three principal components associated with eigenvalues greater than one, which explained 65 % of the variance (see Fig. 3.1). The 'ambulation' variable obtained the highest positive loading on the first principal component, while 'scan' contributed the most to the second principal component (Fig. 3.1a). The third principal component was highly correlated with the proportion of time chipmunks spent in the centre of the arena ('centrality'; Fig. 3.1b).

Mixed model analysis of the open-field tests (121 individuals with one test, 108 individuals with two tests, N = 337) showed that ambulation decreased with time during the open-field test (coefficient $\pm$ SE = - 0.013 $\pm$ 0.001 ; t_1 = 10.67, p <0.001) and between the first and the second trial (trial order, coefficient $\pm$ SE = - 0.411 $\pm$ 0.087, t_1 = 4.71, p <0.001). Ambulation also varied significantly with arena size, year and date (see Annexe 1 for a detailed description of the model). Ambulation level was repeatable (r = 51.26 %, LRT = 279.76, p <0.001). Time spent scanning in the open field (i.e. scan) decreased over time within a given test in the larger arena (time main effect, coefficient $\pm$ SE = - 0.010 $\pm$ 0.004, t_1 = 2.41, p = 0.016), but this effect was null when individuals were tested in the smaller arena (difference in time effect between two arenas : coefficient $\pm$ SE = 0.013 $\pm$ 0.003, t_1 = 4.51, p <0.001). Scan increased more rapidly with time during the first test (coefficient $\pm$ SE = 0.439 $\pm$ 0.149, t_1 = 2.95, p = 0.003) compared to the second test (coefficient $\pm$ SE = - 0.007 $\pm$ 0.002, t_1 = 3.33, p <0.001). Scan also varied with date and year (see Annexe 2). Chipmunks expressed significant individual differences in time spent scanning (r = 27.27 %, LRT = 93.58, p <0.001). Proportion of time spent in the central part of the arena (i.e. centrality) increased with time within a given test (coefficient $\pm$ SE = 0.002 $\pm$ 0.001, t_1 = 2.74, p = 0.006). Centrality also

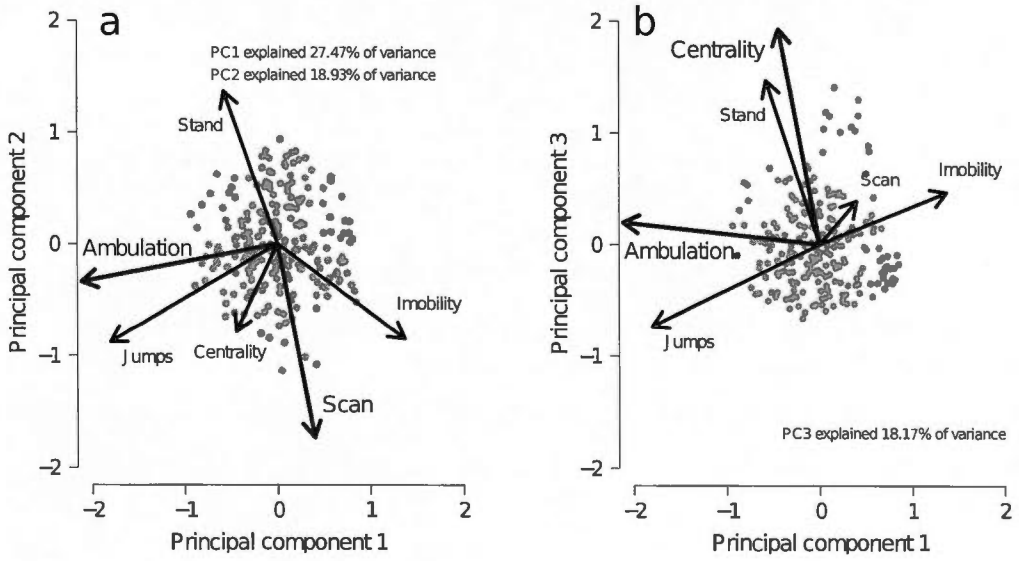

Figure 3.1 (a, b) Principal components (PC) analysis of the behaviour expressed by chipmunks in the open field (N = 78 tests). We selected the behaviour with the highest loading in each of the first three principal components. Behaviours were the number of lines crossed in the arena (ambulation, PC1), the time spent scanning the environment (scan, PC2) and the time spent in the centre of the arena (centrality, PC3). Each open-field test is represented as a grey dot along the principal components. Arrows represent the contribution of each variable to the principal components. Variables retained for all subsequent analyses are shown in red.

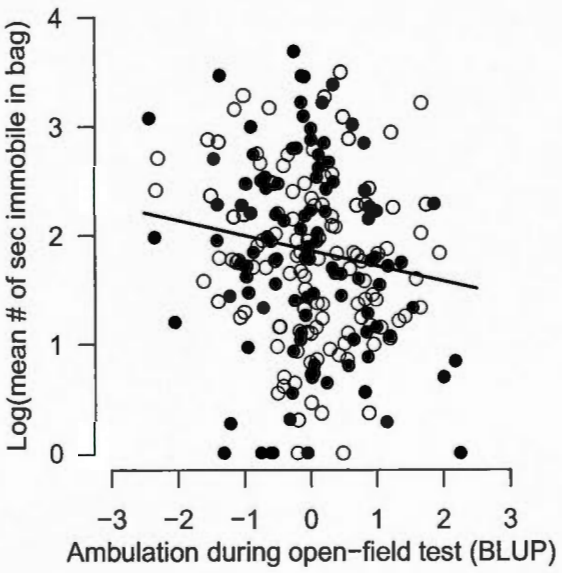

Figure 3.2 Relation between ambulation expressed by female (open dots) and male (filled dots) chipmunks during open-field tests (BLUPs) and mean number of seconds spent immobile in the manipulation bag (N = 4314 tests from 226 individuals).

varied with day and year (see Annexe 3). Chipmunks expressed consistent differences in centrality (r = 19.62 %, LRT = 54.95, p <0.001). All other terms, including the random interaction between individual identity and time interval (within tests) were excluded from the final models.

3.4.2 Bag tests

Time spent immobile in the bag decreased with ambulation in the open field (coefficient ± SE = - 0.144 ± 0.061, t_1 = 2.38, p = 0.018, N = 4314 tests on 226 individuals, number of tests per individual ranged from 10 to 86 ; Fig. 3.2), and increased with trial order (coefficient ± SE = 0.005 ± 0.002, t_1 = 2.55, p = 0.011). Time spent immobile in the bag also varied depending on year and date, but was not affected by centrality or scanning time (Table 3.1). Time spent immobile was repeatable (r = 29, 28 %, LRT = 1049.88, p <0.001).

Tableau 3.1 Final model describing the number of seconds spent immobile by chipmunks during bag tests in our study site from 2005 to 2009. Chipmunk identity was included as a random effect (V_i). BLUPs describing individual differences in behavioural response during open-field tests (ambulation, scan, and centrality) were included as fixed effects (N = 226 chipmunks, 4314 bag tests, LRT = likelihood ratio test, the year 2005 is taken as the reference).

Components	Variance	r	LRT	p
Vi	0.481	29.28 %	1049.88	<0.001
Vr	1.163			
Terms	Coefficient ± s.e.	d.f.	t	p
Intercept	1.860 ± 0.094	1	19.85	<0.001
Year (2006)	-0.276 ± 0.081	1	3.40	0.001
Year (2007)	-0.284 ± 0.101	1	2.82	0.005
Year (2008)	-0.195 ± 0.126	1	1.55	0.120
Year (2009)	0.090 ± 0.146	1	0.62	0.540
Date	-0.006 ± 0.002	1	2.42	0.016
$Date^2$	0.002 ± 0.001	1	4.67	<0.001
Year X date (2006)	0.008 ± 0.003	1	2.72	0.007
Year X date (2007)	0.004 ± 0.003	1	1.42	0.160
Year X date (2008)	0.006 ± 0.003	1	2.33	0.020
Year X date (2009)	0.001 ± 0.003	1	0.36	0.720
trial order	0.005 ± 0.002	1	2.55	0.011
Ambulation	-0.144 ± 0.061	1	2.38	0.018

3.4.3 Probability of capture

Analysis of the 490 trap-days for 66 chipmunks showed that males were less likely to be trapped than females (coefficient ± SE = - 1.254 ± 0.122, z_1 = 10.28, p <0.001). The probability of capture decreased with the distance between the trap and the centre of the home range (coefficient ± SE = - 0.076 ± 0.002, t_1 = 34.01, p <0.001), and this decrease was steeper for females than for males (difference between males and females : coefficient ± SE = 0.030 ± 0.003, t_1 = 9.98, p <0.001). Analysing data for each sex separately (Table 3.2), we found that female trapping distance increased with time spent scanning (coefficient ± SE = 0.021 ± 0.007, z_1 = 3.18, p = 0.001) and time spent in ambulation (coefficient ± SE = 0.007 ± 0.002, z_1 = 2.66, p = 0.007 ; Fig. 3.3a). In males, higher ambulation was related to a higher global probability of capture (coefficient ± SE = 0.140 ± 0.052, z_1 = 2.65, p = 0.008 ; Fig. 3.3b). Males with higher centrality levels were captured closer to the centre of their home range (coefficient ± SE = - 0.052 ± 0.022, z_1 = 2.30, p = 0.021). In contrast to females, trapping distance decreased with scanning time for males (coefficient ± SE = - 0.011 ± 0.004, z_1 = 2.30, p = 0.021).

3.4.4 Faecal cortisol metabolite levels and heart rate measurements

Cortisol concentrations (N = 58 chipmunks, 443 faecal samples) were lower in males than in females (coefficient ±SE = - 0.364 ± 0.158, t_1 = 2.31, p = 0.025). Cortisol level in faeces was negatively related to centrality for females (coefficient ± SE = - 2.830 ± 1.014, t_1 = 2.79, p = 0.007), but positively related to centrality for males (interaction between sex and centrality, coefficient ± SE = 4.732 ± 2.100, t_1 = 2.25, p = 0.028, Fig. 3.4). Faecal cortisol level also varied with the date of sample collection (Table 3.3). Ambulation and scan levels during open-field tests were not related to faecal cortisol levels (p >0.2). However, individuals that displayed lower levels of ambulation in the arena also had higher cortisol variability (coefficient ± SE = - 13.68 ± 4.71, t_1 = 2.90, p = 0.005, Fig. 3.5). Cortisol variability tended to increase as a function of mean faecal cortisol level in samples, but this effect was not significant (p = 0.073). Finally,

Tableau 3.2 Final models describing the probability of capture on the study site in 2008 and 2009 for female (a) and male (b) chipmunks born in 2006 and 2007. Chipmunk identity is included as a random effect (V_i) and the BLUPs describing the behavioural differences expressed by individuals in the open-field test were fitted as fixed effects (ambulation, scan, and centrality, N = 32 females, 34 males, 490 trap-days).

a

Terms	Coefficient ± s.e.	df	z	p
Intercept	0.490 ± 0.094	1	5.22	<0.001
Distance	-0.077 ± 0.002	1	34.53	<0.001
Ambulation	0.122 ± 0.108	1	1.14	0.260
Scan	-0.643 ± 0.289	1	2.23	0.026
Year	0.254 ± 0.108	1	2.35	0.019
Date	0.004 ± 0.001	1	4.43	<0.001
Scan X distance	0.021 ± 0.007	1	3.18	0.001
Ambulation X distance	0.007 ± 0.003	1	2.66	0.008

b

Terms	Coefficients ± s.e.	df	z	p
Intercept	-0.854 ± 0.094	1	9.10	<0.001
Distance	-0.043 ± 0.002	1	22.43	<0.001
Ambulation	0.140 ± 0.052	1	2.65	0.008
Scan	0.344 ± 0.231	1	1.49	0.140
Centrality	2.102 ± 1.124	1	1.87	0.060
Date	0.002 ± 0.001	1	2.52	0.011
Scan X distance	-0.011 ± 0.004	1	2.30	0.021
Centrality X distance	-0.052 ± 0.022	1	2.30	0.021

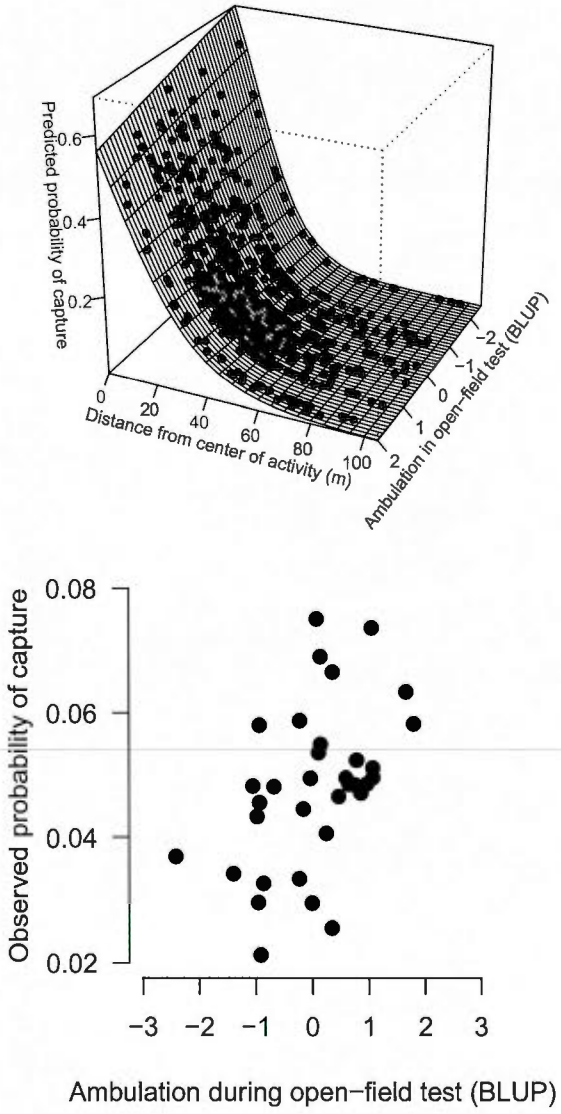

Figure 3.3 (a) Relation between predicted probability of capture, distance from the centre of activity and individual ambulation expressed by female chipmunks during open-field tests (N = 490 trap-days on 32 females). Filled dots represent the trap-days available to estimate the response surface. (b) Relation between ambulation expressed by male chipmunks during open-field tests and observed probability of capture (N = 490 trap-days on 34 males).

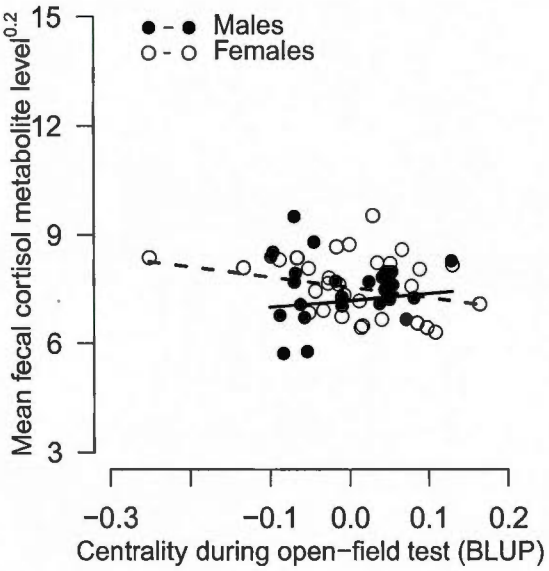

Figure 3.4 Relation between centrality expressed by female (open dots, dashed line) and male (filled dots, solid line) chipmunks during open-field tests (BLUPs) and mean faecal cortisol metabolite level in samples collected in 2009 (N = 443 samples from 58 individuals).

individuals with higher ambulation in the open field expressed a higher heart rate when constrained in the manipulation bag (coefficient $\pm$ SE = 0.216 $\pm$ 0.081, $t_1 = 2.67$, p = 0.009).

3.5 Discussion

In this study, we found behavioural differences among individual eastern chipmunks : individuals varied consistently in their level of ambulation, vigilance and centrality while in a novel environment. We also found that exploration in the open-field arena (measured as ambulation level) was associated with docility when handled and with the propensity to enter traps, often taken as an index of boldness (Wilson, 1998; Réale et al., 2000; Boon, Réale et Boutin, 2007). Individuals with a slower exploration pattern in open-field tests also displayed a lower reactivity of their sympathetic autonomic system but a more variable faecal cortisol level over the course of their active season. We first

Tableau 3.3 Final model describing the cortisol metabolite level in faecal samples collected at the study site in 2009. BLUPs describing the individual behaviours expressed during open-field tests (ambulation, scan and centrality) were included as fixed effects. Chipmunk identity was included as a random effect (V_i, N = 58 chipmunks, 443 samples, LRT = likelihood ratio test, female is taken as the reference).

Components	Variance	r	LRT	p
Vi	0.001	0.00 %	0.001	1.00
Vr	2.413			

Terms	Coefficient ± s.e.	d.f.	t	p
Intercept	7.551 ± 0.093	1	81.63	<0.001
Date	0.015 ± 0.002	1	8.48	<0.001
Centrality	-2.830 ± 1.014	1	2.79	0.007
Sex (Male)	-0.364 ± 0.158	1	2.31	0.025
Centrality X sex (Male)	4.732 ± 2.100	1	2.25	0.028

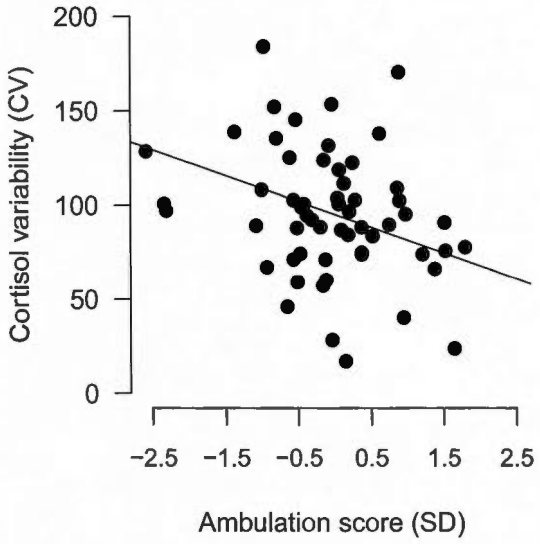

Figure 3.5 Relation between ambulation in open-field tests (BLUPS) expressed as standard deviations (SD) and within-individual coefficients of variation (CV) in faecal cortisol levels (%, N = 438 samples from 57 individuals).

discuss the relationship between exploration, docility and trappability from an ecological perspective, then their neuroendocrinological correlates.

3.5.1 Behaviour in the open-field arena

The open-field tests were conducted to quantify behavioural responses of chipmunks to a novel environment. Most of the variation in behaviour in the open field was associated with the level of ambulation, and individuals differed consistently in this behaviour. While individual differences in exploration have been reported in many study systems (Réale et al., 2007), very few studies have documented consistent differences over the lifetime of individuals in the wild. The long timescale over which these differences were measured in our population may also explain why our effect sizes were small. However, such effects are still relevant considering that they were maintained over the lifetime of individuals under varying environmental conditions. In rodents, consistent differences in ambulation in a novel arena may have implications for the ecology and fitness of in-

dividuals, as this behaviour is often associated with exploration (Archer, 1973), better spatial memory, or spatial cognitive skills (Jozet-Alves, Modéran et Dickel, 2008). In many rodent species, these traits strongly affect foraging, mating and escaping predators (Galea, Kavaliers et Ossenkopp, 1996). Consistent differences in ambulation may also have other implications as chipmunks with higher ambulation levels in the arena also struggled more when handled and were trapped more frequently (males) or farther from their burrows (females). Differences in docility suggest that chipmunks with lower exploration levels in the arena may respond differently to the presence of human activity (e.g. tourism) in their environment. For example, Martin & Réale (2008) reported that chipmunks with burrows located farther from the trails used by tourists displayed lower levels of exploration during open-field tests. In our study, faster-exploring males may have a higher probability of capture because they spend more time out of their burrows. In contrast, we found that faster-exploring females in the arena were captured farther from the centre of their home range. Again, a trap may be seen as an artificial food source in an otherwise natural environment. In such a case, differences in trappability would suggest that individuals with higher levels of exploration may have a higher capacity to exploit artificial anthropogenic food sources. Additionally, the differences in trapping propensity or in distance between trapping location and burrow may modify the susceptibility of individuals to parasites (Boyer et al., 2010; Patterson et Schulte-Hostedde, 2011). Investigating how exploration levels in the open-field arena is representative of differences in space use patterns is beyond the scope of this study, but warrants further investigation.

3.5.2 Physiological correlates of chipmunk exploration

Faster explorers showed a greater increase in heart rate during a restraint test, which suggests greater reactivity of the sympathetic nervous system. The autonomic system is often presented as the mechanism for the 'fight or flight' response of individuals, activated instantly when facing environmental perturbations. A classical view is that this response is beneficial for an animal's fitness, by activating all the organism's functions

that are directly associated with immediate survival. However, the fitness implications of such individual differences in natural populations remain to be tested.

Our study also investigated the relationship between consistent individual differences in exploration and the faecal cortisol levels of individuals. Fecal cortisol (or corticosterone in birds) metabolite level represents an average measure of cortisol levels secreted into the blood over a period of about 10 h (Palme, 2005; Montiglio et al., 2012b) and may also reflect the capacity of the individual to mobilize cortisol when facing an environmental perturbation (Sheriff, Krebs et Boonstra, 2010). In our study, less central females displayed higher mean faecal cortisol levels. This result is in accordance with the relationship found between exploration and blood cortisol in other species (Carere et al., 2003; Baugh et al., 2012). In contrast to females, faecal cortisol level was not associated with male behaviour in the open-field arena. We showed that these measures were not repeatable for individuals in natural conditions, which may explain the rather weak relationship reported here.

Repeated measurement of faecal cortisol levels over an entire season allowed us to investigate the variability of cortisol levels, an aspect that is seldom documented in wild animals. Individuals with lower ambulation levels in the open field also displayed higher variability in their faecal cortisol level over a complete active season. Indeed, we hypothesized that slower explorers should display a higher reactivity of the hypothalamic-pituitary-adrenal axis (Koolhaas et al., 1999). Higher short-term cortisol secretion in response to environmental perturbations has been shown to translate into a higher daily cortisol variability in humans (Corbett et al., 2009), but has never been investigated in a wild animal population to our knowledge. An alternative explanation could be that slower explorers experience habitats with a higher rate of short-term stressors or less predictable variations. In captive black, *Diceros bicornis*, and white rhinoceroses, *Ceratotherium simum*, higher rates of agonistic interactions and exposure to humans are associated with higher cortisol variability in males and females (Carlstead et Brown, 2005). In these animals, higher cortisol variability is associated with higher mortality rate in both sexes and with disturbed oestrous cycles in females. At present, little is

known about the fitness consequences of higher variability of cortisol levels in nature.

3.6 Conclusion

In conclusion, our study shows that the open field quantifies three main components of chipmunk behaviour : exploration, vigilance and centrality. We showed that fast explorers were less docile and bolder, and displayed greater sympathetic autonomic system reactivity. We followed cortisol levels of individuals over several months and found that exploration was not associated with mean cortisol level (which was not repeatable), but was instead related to the variability of cortisol levels. Overall, our study presents the eastern chipmunk as an interesting model species for the study of individual differences in behaviour and physiology. Future studies analysing how exploration differences affect ecology and fitness of individuals will surely provide fruitful insights into the evolutionary mechanisms responsible for variation in exploration, docility, boldness and stress physiology within animal populations.

3.7 Acknowledments

We thank the Ruiter Valley Land Trust for access to study site, members of the Sutton chipmunk project, Hélène Presseault-Gauvin for valuable help with the faecal cortisol analyses, and three anonymous referees for constructive comments on an earlier draft. P.-O.M was supported by a grant from the Fonds de recherche du Québec-Nature et technologies (FQRNT). D.R., D.G. and F.P. acknowledge the Natural Sciences and Engineering Research Council (NSERC) of Canada for Discovery grants and Canada Research Chairs to D.R. and F.P. We declare no conflict of interest.

CHAPITRE IV

PULSED RESOURCES AND THE COUPLING BETWEEN LIFE-HISTORY STRATEGIES AND EXPLORATION PATTERNS IN EASTERN CHIPMUNKS (*TAMIAS STRIATUS*)

Pierre-Olivier Montiglio, Dany Garant, Patrick Bergeron, Gabrielle Dubuc Messier & Denis Réale

4.1 Abstract

Understanding the causes of consistent behavioural differences, expressed across contexts, or animal personality, is a major aim of evolutionary studies. Alternative life-histories and fluctuations in resources could explain the occurrence of such consistent behavioural differences, yet studies of their relationships in wild populations remain scarce. We used a long-term survey on a wild population of eastern chipmunks to investigate the relationships between reproductive life-history and consistent individual differences in exploration. Eastern chipmunks may breed for the first time at 24, 33 or 50 % of their average lifespan, depending on food abundance and season of birth. Individuals with a more thorough exploration pattern displayed a higher fecundity (females) or a higher fertilization success (males) later in their life than superficial explorers. Overall, thorough exploring males and females attained a higher lifetime reproductive success than superficial explorers when given an opportunity to reproduce later in their life. Our results suggest that the timing of mating seasons, associated with pulsed fluctuating food abundance, may favour different exploration patterns, as a personality trait,

through its effects on reproductive life-history.

4.2 Introduction

Animals often differ consistently in their behaviours through time (i.e. the so-called animal personality traits). For example, some animals in a population may be consistently more active than others (Sih, Kats et Maurer, 2003). Individuals will often also exhibit consistent relationships between their behaviour expressed in different contexts, a feature called behavioural syndromes (Bell, 2005). The study of ecological and evolutionary consequences of animal personality and behavioural syndromes is now a highly dynamic area of research (Sih et Bell, 2008; Réale et al., 2010a). Animal personality traits and behavioural syndromes were originally studied to assess their potential effects on ecological and evolutionary processes (Sih, Bell et Johnson, 2004; Réale et al., 2007). However, since these two phenomena have also been showed to exhibit significant levels of additive genetic variation (Dingemanse et al., 2002; Réale et Festa-Bianchet, 2003; Bell, 2005), the focus of research studies has more recently shifted to understanding the adaptive causes of animal personality and behavioural syndromes (Réale et al., 2010a; Dingemanse et Wolf, 2010).

Theoretical studies suggest that consistent individual variation in behaviour is associated with individual variation in life history traits, because both behavioural and life history traits contribute to evolutionary trade-offs (Réale et al., 2010a). Indeed, differences in aggressiveness, boldness or exploration could incur costs in terms of mortality but enable individuals to express an earlier reproduction or a higher growth rate or fecundity (Stamps, 2007; Biro et Stamps, 2008). If some individuals in a population put more emphasis on early reproduction, growth and fecundity than on survival (Bielby et al., 2007), we may expect them to be more aggressive, bolder or faster explorers (Clark, 1994; Réale et al., 2010a). The empirical studies available so far suggest that more aggressive, bolder or fast exploring individuals usually display lower survival in natural environments (Smith et Blumstein, 2008). These individuals also seem to have a higher resource acquisition potential (Biro et Stamps, 2008) and tend to be favoured in

conditions of high resource availability (Dingemanse et de Goede, 2004; Boon, Réale et Boutin, 2007). For example, decreasing the survival expectations of individual great tits (*Parus major*) affected their exploration behaviour : individuals with decreased survival expressed faster exploration patterns when tested in a novel environment, suggesting that individuals may plastically adjust their behaviour to their life history expectations (Nicolaus et al., 2012).

Personality traits could also co-evolve with life history traits through their effect on the trade-off between early and late reproduction, as opposed to the one between reproduction and survival, (Williams, 1966). Individuals putting more emphasis on early reproduction would invest more importantly in behaviours associated with immediate resource acquisition (i.e. boldness and aggressiveness) but less in behaviours having immediate costs in time and energy and future benefits in terms of reproduction. For example, Wolf et al. (2007) presented a model suggesting that individuals investing more in exploring their environment should incur immediate costs in terms of reproductive success but, on the other hand, exploration should be beneficial for future reproduction. If such an assumption is verified, one should expect a negative relationship between early reproduction and exploration (Wolf et al., 2007; Réale et al., 2010a). This prediction is opposite to the idea presented in the previous paragraph (i.e. exploration is risky) and predicts a positive relationship between 'fast' exploration and fecundity, or even early reproduction. Unfortunately, investigating the relationships between age, reproduction and personality may be obscured by the selective disappearance of individuals, or by individual heterogeneity (Nussey et al., 2006; Weladji et al., 2006) and requires longitudinal data sets, following the personality and reproduction of individuals over their life span (Nussey et al., 2006; van de Pol et Verhulst, 2006). Studies using such data sets and quantifying the relationship between age, reproduction and personality at the individual level remain scarce (Boon, Réale et Boutin, 2007; Quinn et al., 2009).

A co-evolution between personality and life history also predicts that the personality traits associated with life history strategies will form behavioural syndromes (Wolf et al., 2007; Wolf, van Doorn et Weissing, 2008). Hence, if aggressiveness, boldness and explo-

ration are all associated with a faster life history, these behaviours should be correlated. Such behavioural syndromes are found in a number of study systems (Bell, 2005; Dingemanse et al., 2007). However, we also expect that personality traits will form a stronger behavioural syndrome as individual variation in life history increases, as the strength of the relationship should increase with an increasing variance in personnality and life history. Hence, the strength of behavioural syndromes may fluctuate over time in populations where the variation in life history changes through time. This may happen when individuals from different birth cohorts (Sheriff, Krebs et Boonstra, 2010) or different habitats (Blondel, 2007) display differing life histories. While some studies previously investigated behavioural syndromes in different populations (Bell, 2005; Dingemanse et al., 2007; Dingemanse et al., 2012; von Merten et Siemers, 2012), more studies investigating how individual variation in life history predict behavioural syndromes within populations over time are needed.

In this study, we use a long-term survey of a wild population of eastern chipmunks (*Tamias striatus*) in southern Québec to investigate the relationships between individual variation in life history and behaviour. In this population, individuals show consistent differences in their exploration patterns, which range from thorough, characterized by a moderate but constant level of activity in a novel environment, to superficial, characterized by a high initial activity and a steep decrease throughout the test (Montiglio et al., 2010). Chipmunk survival and reproduction are tightly associated with the availability of American beech (*Fagus grandifolia*) seeds, their main food resource, which exhibit mast events every 2 - 3 years (Bergeron et al., 2011a). During a mast year characterized by high food abundance, chipmunks breed in the summer, and juveniles are weaned by the end of August at the time of maximum food availability. They also breed in the following spring, juveniles being weaned by the end of June. As a result, individuals born in the summer may breed for the first time in spring or summer following their birth at 7 or 10 months depending on the year. In contrast, individuals born in the spring may only reproduce for the first time the next spring at 15 months of age. Individuals born in the spring or the summer that did not succeed in reproducing are constrained to

delay their first reproduction as late as 23 months of age (Fig. 4.1a, see also Figure 1 in Bergeron et al. 2011b). Given that a chipmunk average lifespan in that population is 30 months, birth cohort and thus mast event strongly shape the reproductive life history of individuals (Bergeron et al., 2011a).

First, we investigated the relationship between age at first reproduction and exploration pattern at the population level to test for the presence of a relationship between life history and personality. We recently showed that superficial and thorough explorers have a higher survival than individuals with average exploration patterns (Bergeron et al., 2013). Based on the assumption that exploration is incurring immediate reproductive costs, we expect that thorough exploration should be related to a delayed age at first breeding, compared to superficial exploration. Second, we investigated the relationship between age-specific reproductive success and exploration patterns. More superficial explorers should display a higher early reproductive success while thorough explorers should display a higher reproductive success later in their life. Third, we tested if the timing of the mating seasons could be associated with the maintenance of individual variation in exploration patterns by investigating whether an earlier opportunity to reproduce (associated with summer birth cohorts) increases the reproductive success of more superficial explorers. Fourth, we investigated the correlation between exploration pattern and docility toward human handlers in each sex and birth cohorts to test whether the magnitude of individual variation in life history among individuals within a birth cohort is associated with the strength of a behavioural syndrome between exploration and dociliy in this population. Along the studies available on rodents (Koolhaas et al., 1999; Boon, Réale et Boutin, 2007), we expected a negative relationship between exploration superficiality and docility. We expected to detect stronger correlations between exploration and docility in the summer birth cohorts, where individuals vary more extensively in their age at first reproduction compared to the spring birth cohorts where almost all individuals reproduce at the same age (Fig. 4.1 and methods section).

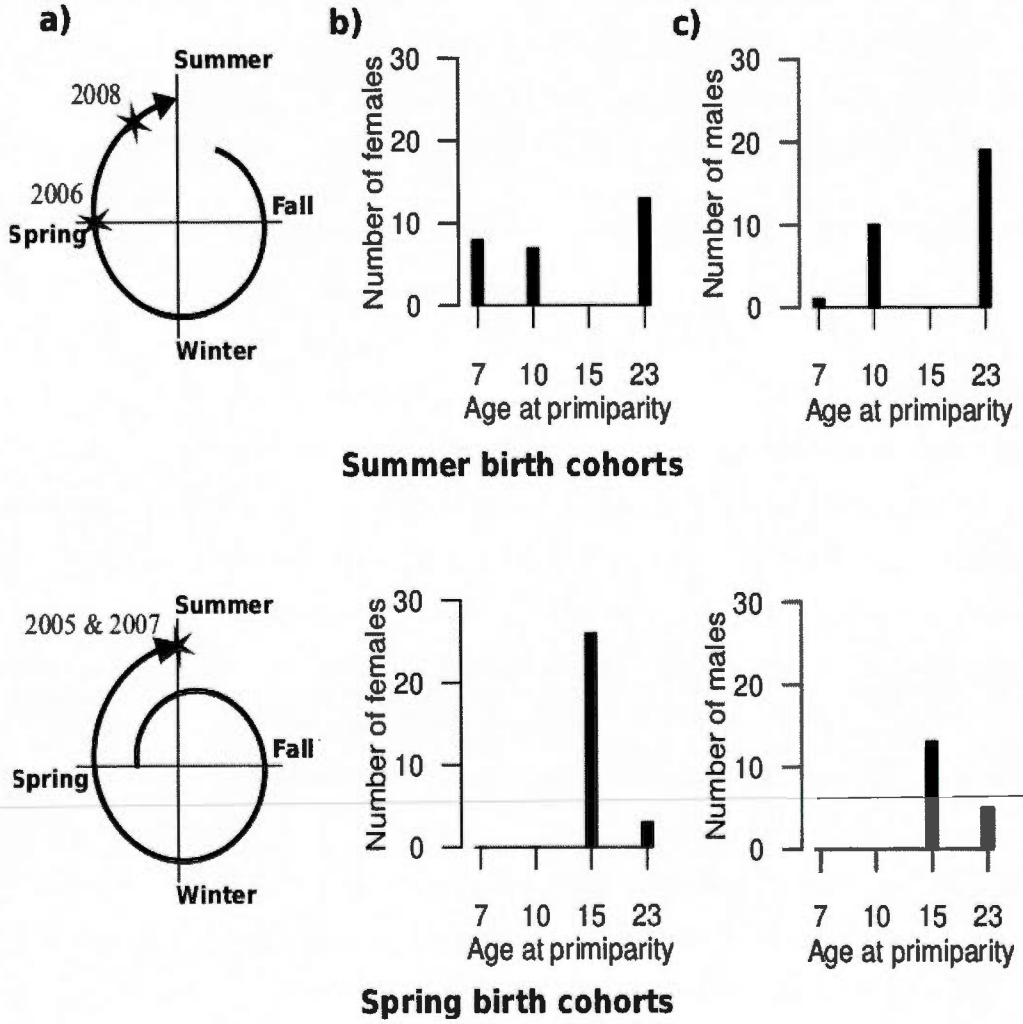

Figure 4.1 Reproduction patterns in the studied eastern chipmunks population. Panel (a) represents the life-history from birth (beginning of line) to the first opportunity for reproduction (stars) of individuals born in the summer 2006 and 2008 (upper part) or in the spring 2005 and 2007 (lower part). Individuals may reproduce for the first time at 7 months of age (males and females born in summer 2006, males born in the summer 2008), 10 months of age (females born in the summer 2008) or 15 months of age (males and females born in the spring 2005 and 2007). Individuals that failed to reproduce at their first opportunity delayed reproduction to 23 months of age. Panels b and c show the number of females and males mating for the first time at each age, respectively.

4.3 Materials and methods

4.3.1 Study system and site

We studied eastern chipmunks on a 25 ha grid, in the mounts Sutton, southern Québec, Canada (45°05' N, 72°25' W) from 2005 to 2009. Eastern chipmunks are ground dwelling Sciurids feeding mainly on seeds from masting trees such as maple *Acer sp* or beech *Fagus sp* (Landry-Cuerrier et al., 2008). During the winter they rely on seeds hoarded in their burrow and exhibit torpor to save energy (Snyder, 1982). In the mounts Sutton population, chipmunks usually display two mating seasons associated with a mast. The first mating season occurs in June while a second mating season occurs in March of the following year and produces juveniles that are weaned during the summer (Bergeron et al., 2011a). Mating seasons last for around three weeks (Bergeron et al., 2011b). A female oestrus lasts for a day during which the female can copulate with several males. This situation generates important competition between males for the access to and the fertilization of females (Elliott, 1978). Females give birth to 2 to 8 pups that spend the first 4 to 5 weeks of their life in the maternal burrow before emerging above ground and dispersal (Elliott, 1978; Snyder, 1982).

Each year from May to October, we conducted daily trapping sessions to systematically mark, sex, weigh and sample individuals for DNA on a 25 ha grid using >250 traps (Chambers et Garant, 2010). We systematically trapped individuals over the whole grid each week. We also assessed the reproductive status of females by examination of nipple and vulva size (Bergeron et al., 2011b). Most individuals were fitted with radio transmitters to locate their burrow (4g, model PD-2C, Holohil Systems Ltd, Ontario). Trapping session targeting females with signs of pregnancy as well as frequent observations directly at their burrow allowed us to capture most juveniles at their maternal burrow before dispersion (Bergeron et al., 2011b). We thus assumed that females with no observed litter during the study did not succeed at weaning any juvenile. We considered that all individuals marked before the reproductive season of 2006 were from the 2005 birth cohort. In this population, most adults died after two years, although a

few individuals can survive on the grid for up to 5 years (Bergeron et al. unpublished results). Rerunning all analyses described in this study without individuals of unknown age yielded similar estimates. We assigned paternity of juveniles using 11 polymorphic microsatellite loci previously used in this population (Chambers et Garant, 2010; Bergeron et al., 2011b). We used the software CERVUS 3.0.3 (Kalinowski, Taper et Marshall, 2007) along with prior information on mother-offspring association at the burrow entry to assign the most likely father to each juvenile with a 95 % confidence level. All the males trapped on the study site during a year were considered as potential fathers. We assigned paternity to 75 % of the juveniles with a known mother. Thus, our data on male reproduction was limited compared to data on female reproduction. Assignation effort and success were lower in 2006 and 2007 (25 juveniles assigned out of 40) compared to 2008 and 2009 (146 juveniles assigned out of 187). This reduced our capacity to determine male reproductive success in 2006-07, which likely limited our statistical power for those years. We thus ran analyses successively with and without data from 2006-07. Analyses using the restricted data set yielded similar effect sizes than analyses based on the whole data set (results not shown) and hence we are confident that this limitation does not affect the validity of our results. However, because our capacity to assign paternities may limit our statistical power for males, and because relationships between personalities and life-history strategies may differ between sexes, we analyzed males and females separately throughout this paper.

Because of the timing of mast events and mating seasons, individuals from the summer birth cohorts (mast years) experienced their first opportunity of reproduction the next spring, in March, at 7 (males and females from the 2006 birth cohort, males from the 2008 birth cohort) or 10 (females from the 2008 birth cohort) months of age (see Fig. 4.1a). In contrast, individuals born in the spring 2005 and 2007 experienced their first opportunity of reproduction only during the summer, in June, of the next year at 15 months of age (Fig. 4.1a). Summer and spring-born individuals that missed the first breeding opportunity were constrained to delay their first reproduction to the following summer, at 23 months of age (Fig. 4.1b). We therefore divided all reproductive males

and females as early breeders (i.e. 7 months of age), intermediate female breeders (i.e. 10 months of age), late breeders (i.e. 15 months of age) and very late breeders (23 months of age). Note that we distinguish between age at first opportunity to reproduce, which occurred at 7, 10 or 15 months, and actual age at first reproduction, which we present as early, intermediate, late and very late, throughout this study. Individuals with an opportunity to reproduce at 7, 10 or 15 months of age may still reproduce successfully for the first time at 23 months of age.

4.3.2 Exploration and docility measurements

We conducted open-field tests to quantify the behavioural response of individuals to a novel environment (Montiglio et al., 2010). The tests were conducted directly on the study site, between May and October each year. Upon capture, individuals were carried in their trap to the arena, located at the center of the trapping grid. Individuals were then transferred without manipulation to a mesh bag and to a dark chamber connected to the arena. We then delicately pushed the individuals inside the arena and blocked the entrance with a plastic sliding panel. Individuals were then videotaped for 90 seconds. In these tests, chipmunks have been shown to display consistent differences in their exploration patterns : while, at one extreme, some individuals begin the test with a high level of exploration but decrease substantially their exploration during the following seconds, some other individuals display more moderate but constant exploration levels throughout the test. Relying on the assumption that exploration in this case provides chipmunks with information on their environment (Wolf et al., 2007), we labelled these two extreme exploration patterns superficial and thorough respectively. We tested individuals born on the grid upon emergence from their maternal burrow ($\sim$ 56 % of the population). Individuals born in 2005 and immigrants were tested as adults. Some of these individuals were then tested a second time the following year ($\sim$ 40 % of the individuals previously tested). We analyzed the distance covered by individuals during open-field tests using linear mixed model, which enabled us to compute an exploration score for each individual (Best Linear Unbiaised Predictors) while correcting for envi-

ronmental effects such as age, date, year, sex, and trial order (Montiglio et al., 2010). We measured docility of individuals during each capture by transferring the animal from the trap to a mesh bag and counting the number of seconds spent immobile within a minute prior to any manipulation (Montiglio et al., 2012a).

4.3.3 Statistical analyses

Exploration and age at first breeding

For each sex, we analyzed the relationship between individual exploration pattern and age at first breeding (early, intermediate, late, and very late) using a linear model. An important proportion of individuals failed to reproduce (see Results section and figure 3.4). We thus chose to restrict these analyses to individuals known to have produced at least one juvenile during their lifetime.

Exploration and age-specific reproductive success

We analyzed the overall relationship between litter size at weaning and exploration of all females known to have produced at least one juvenile over their lifetime using a linear mixed effect model. We included age, individual exploration pattern, mating season (spring or summer) in the model using a Gaussian distribution. Because we were specifically interested in the age related reproductive success of individuals as a function of their exploration pattern, we fitted an interaction between exploration and age. Similarly, we tested whether the spring and fall mating seasons favoured individuals with particular exploration patterns by adding an interaction between exploration pattern and season. Finally, because reproductive success often decreases with advancing age, we added a nonlinear quadratic age effect to our model. To avoid pseudo replication, we added female identity as a random effect. The number of juveniles sired by a male followed a Poisson distribution with important under-dispersion. We thus analyzed the first successful reproduction of each male using a generalized linear model with a Quasi-Poisson distribution (GLM, one observation per male). We added the same explanatory

variables as for females.

Effect of age at first breeding opportunity and exploration pattern on reproductive success

Female reproductive success followed a non-normal distribution. To ascertain our results on reproductive success, we thus built two models examining the effect of age at first breeding opportunity on a) litter size per breeding event, and b) the probability of weaning at least one juvenile (binary variable) using generalized linear mixed effect models with Gaussian (a) and binomial (b) distributions respectively. We included age at first breeding opportunity (i.e. 7, 10 or 15 months of age), individual exploration pattern, mating season (spring or summer) and the interaction between exploration and age at first breeding opportunity as fixed effects. We also tested whether the fall and spring seasons favoured individuals with different exploration patterns by fitting an additional interaction between exploration pattern and season. We also included female identity as a random effect. We analyzed male lifetime reproductive success and lifetime probability of siring at least one juvenile as a function of age at first opportunity for reproduction in a similar way by using a generalized linear model with a Quasi Poisson distribution (lifetime reproductive success) or a Quasi binomial distribution (probability of siring at least a juvenile) to account for the under-dispersion of male reproductive success.

Relationship between exploration and docility

For each sex, we assessed how the amount of individual variability in life history predicted the strength of the relationship between docility and exploration. We ran two separate mixed models with an index of docility (log transformed number of seconds spent immobile in handling bag) as the response variable for either individuals born in the summer (i.e. cohorts with higher variation in life history, composed of early, intermediate or very late breeders) or individuals born in the spring (i.e. cohorts with lower variation in life history, composed of late and very late breeders). Since we were

only interested in the relationship between exploration and docility, we only included individual exploration pattern in the open-field as an explanatory variable (fixed effect). We also included individual identity as a random effect.

We ran all models using R 2.15.2 (R Development Core Team 2012). Generalized linear models were fitted using the *glm* function available from the *stats* package in R (R core development team 2012). Linear mixed models and generalized linear mixed models were fitted with the functions *lme*, from the package *nlme* (Pinheiro et Bates, 2000) and *glmer* from the package *lme4*, (Bates, Maechler et Bolker, 2011), respectively. We simplified all the models described above by stepwise deletion of all non-significant terms (Crawley, 2007). Model simplification using AIC (when applicable) yielded similar results (result not shown). We inspected the final models visually for homoscedasticity and normality of residuals, and obtained similar results when using rank transformed data, indicating that the patterns reported are robust. Estimates are presented with standard errors (SE).

4.4 Results

4.4.1 Exploration and age at first breeding

Females bred on average at 15 months of age (estimate from a linear model : 15.84 $\pm$ 0.90, $t_{1,50}$ = 17.53, p <0.001). Females breeding earlier tended to express a more superficial exploration pattern (-1.910 $\pm$ 1.140, $t_{1,50}$ = 1.64, p = 0.10, adjusted r^2 = 0.03, N = 52 females). In fact, omitting a female outlier (with the highest residual and an important leverage) resulted in a significant negative relationship (-2.740 $\pm$ 1.220, $t_{1,49}$ = 2.24, p = 0.029). Males bred on average at 18 months of age (18.45 $\pm$ 0.90, $t_{1,52}$ = 20.45, p <0.001). Age at first reproduction was not significantly related to exploration pattern in males (1.355 $\pm$ 0.937, $t_{1,52}$ = 1.44, p = 0.15, N = 52 males).

Tableau 4.1 Final linear mixed model analyzing female litter size as a function of age and exploration pattern. We kept only females known to have produced a juvenile during their lifetime (N = 43 females, 123 seasons, LRT = likelihood ratio test, d.f. = degrees of freedom, the fall season is taken as the reference).

Components	Variance	LRT	d.f.	p
Vi	0.310	0.415	1	0.520
Vr	1.257			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	0.825 ± 0.519	1.62	1 (76)	0.110
Exploration pattern	0.476 ± 0.277	1.71	1 (41)	0.093
Age	0.114 ± 0.044	2.58	1 (76)	0.012
Age^2	-0.002 ± 0.001	2.61	1 (76)	0.011
Season	-1.28 ± 0.23	5.43	1 (76)	<0.001
Exploration X age	-0.035 ± 0.012	2.83	1 (76)	0.006

4.4.2 Exploration and age-specific reproductive success

Females with a more superficial exploration pattern produced larger litter sizes earlier in their reproductive lifetime, as shown by an interaction between exploration and age ($F_{1,76} = 8.02$, $p = 0.006$, N = 123 mating events from 43 females, number of observations per females ranged from 2 to 5, mean = 2.86, see Fig. 4.2 and Table 4.1 for the estimates from the final model). The quadratic age effect was also retained in the final model ($F_{1,76} = 6.82$, $p = 0.011$). Females also produced smaller litter sizes during the spring seasons ($F_{1,76} = 29.53$, $p < 0.001$). Mating season did not interact with age or exploration (all $p > 0.5$).

In males, the probability of siring at least one juvenile during the first opportunity for reproduction increased with age (0.290 ± 0.098, $t_{1,60} = 2.95$, $p = 0.004$, N = 62, one observation per male, dispersion parameter = 0.011), but was not related to exploration

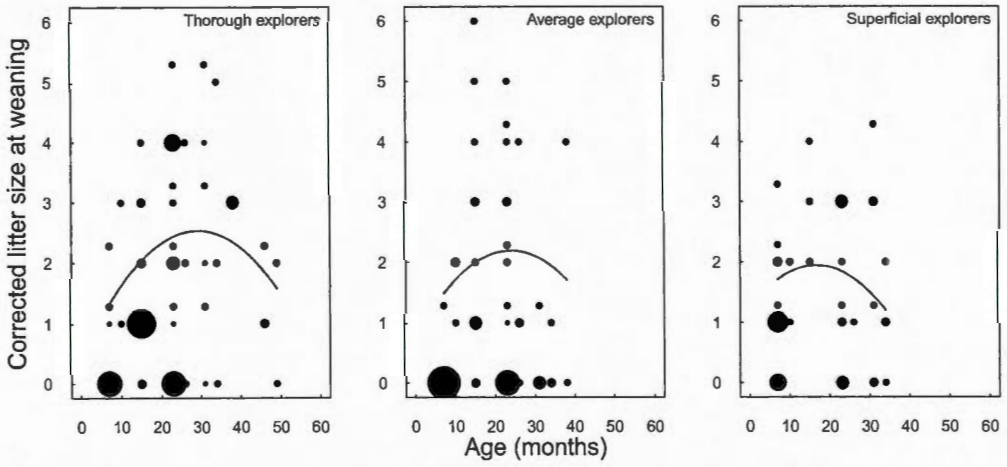

Figure 4.2 Litter size as a function of age (in months) for female eastern chipmunks, from 2006 to 2009. Litter size was corrected for the mating season. The left panel shows thorough exploring females (the lowest third of the distribution of exploration patterns), centre panel shows females with average exploration patterns (between lowest and the highest thirds of the distribution of exploration patterns) and right panel shows superficial exploring females (highest third of the distribution of exploration patterns). The diameter of the dots is proportional to the number of observations (123 litters from 43 females). The effect sizes were computed from a model including age, exploration pattern, season in addition to an interaction between age and exploration pattern and a quadratic effect of age. Female identity was included as a random effect.

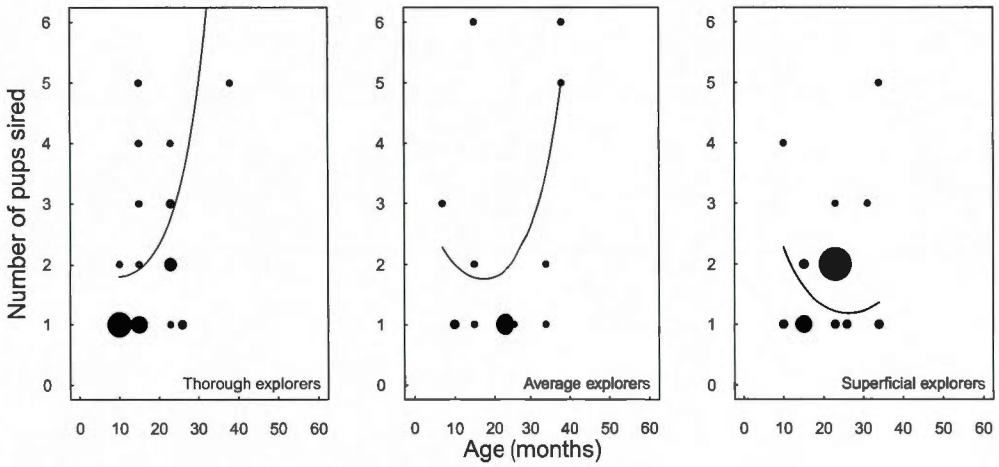

Figure 4.3 Number of juveniles sired (paternity) as a function of male age (in months) in eastern chipmunks, between 2006 and 2009. The left panel shows thorough exploring males (the lowest third of the distribution of exploration patterns), the centre panel shows males with average exploration patterns (between lowest and the highest thirds of the distribution of exploration patterns) and the right panel shows superficial exploring males (highest third of the distribution of exploration patterns). The diameter of the dots is proportional to the number of observations (N = 52 males). The effect sizes were computed from a model including age, exploration pattern and their interaction. Male identity was also included as a random effect.

pattern (excluded from the final model, p >0.5). Number of juveniles sired increased as a function of age in thorough explorers while it decreased for superficial explorers (when analyzing the number of juveniles sired by males that succeeded at siring at least one juvenile, interaction exploration by age : -0.044 $\pm$ 0.0180, $t_{1,48} = 2.44$, p = 0.018, N = 52, one observation per male, see Fig. 4.3 and Table 4.2 for estimates). A positive quadratic effect of age was also retained in the final model (0.002 $\pm$ 0.001, $t_{1,48} = 3.16$, p = 0.003). Mating season was excluded from the final model (p = 0.09).

Tableau 4.2 Final generalized linear model analyzing the number of juvenile sired by males that sired at least one juvenile during their lifetime (N = 52, residual deviance = 0.29 on 48 degrees of freedom, d.f. = degrees of freedom).

Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	1.334 ± 0.402	3.32	1	0.002
Exploration pattern	0.571 ± 0.333	1.71	1	0.093
Age	-0.087 ± 0.037	2.37	1	0.021
Age^2	0.002 ± 0.001	3.16	1	0.002
Exploration X age	-0.044 ± 0.018	2.44	1	0.018

4.4.3 Effects of age at first breeding opportunity and exploration on reproductive success

Among females with their first opportunity for reproduction at 7 months of age, females with more superficial exploration patterns had a higher chance of weaning at least one young (0.725 ± 0.379, z_1 = 2.04, p = 0.041, N = 43 females, 2.86 mating seasons per female, see Table 4.3). In contrast, among females with a first opportunity for reproduction at 10 months, chance of weaning at least one young tended to be negatively related to exploration pattern (-1.050 ± 0.577, z_1 = 1.82, p = 0.067). This effect was even stronger among females with a first opportunity for reproduction at 15 months of age (-2.100 ± 0.593, z_1 = 3.53, p <0.001, N = 97, 1.93 mating seasons per female, Fig. 4.4). Analyzing the number of juveniles sired instead of the probability of weaning showed that exploration pattern did not affect the number of juveniles weaned by females with a first opportunity to reproduce at 7 months (0.077 ± 0.250, z_1 = 0.31, p = 0.756, see Table 4.4) or at 10 months (-0.238 ± 0.401, z_1 = 0.59, p = 0.552). However, among females with a first opportunity to reproduce at 15 months, more superficial exploration pattern was related to a lower number of juveniles weaned (-0.735 ± 0.360, z_1 = 2.03, p = 0.041). Females breeding during the spring weaned fewer juveniles than females

Tableau 4.3 Final generalized mixed model analyzing the female probability of weaning at least one juvenile (N = 97 females, 188 mating seasons, residual deviance = 212.71 on 180 degrees of freedom, LRT = likelihood ratio test, d.f. = degrees of freedom, the fall season is taken as the reference).

Components	Variance	LRT	d.f.	p
Vi	0.261	0.171	1	0.680
Vr	0.511			

Terms	Coefficient ± s.e.	t	d.f.	p
Age at first reproduction (7 months)	0.177 ± 0.467	0.38	1	0.700
Age at first reproduction (11 months)	-0.351 ± 0.620	0.56	1	0.570
Age at first reproduction (15 months)	0.277 ± 0.399	0.69	1	0.490
Exploration (7 months)	0.775 ± 0.380	2.04	1	0.041
Exploration (11 months)	-1.050 ± 0.577	1.82	1	0.067
Exploration (15 months)	-2.100 ± 0.593	3.53	1	<0.001
Season	-1.656 ± 0.578	2.85	1	0.004

breeding during the summer (-1.150 ± 0.199, $z_1 = 5.76$, p <0.001).

When considering all the males present on our study site, including males for which we had no evidence of reproduction, we found no relationships between male lifetime probability of siring at least one offspring and either age at first breeding opportunity or exploration pattern (all p >0.4, N = 90 males). Among the males who sired at least one juvenile during their life, exploration pattern did not affect male lifetime reproductive success among males with a first opportunity to reproduce at 10 months of age (0.246 ± 0.147, $t_{1,48} = 1.62$, p = 0.11). In contrast, among males with a first opportunity to reproduce at 15 months, males with more thorough explorers significantly sired more juveniles over their lifetime (-0.523 ± 0.210, $t_{1,48} = 2.49$, p = 0.016, N = 54, Fig. 4.5 and Table 4.5).

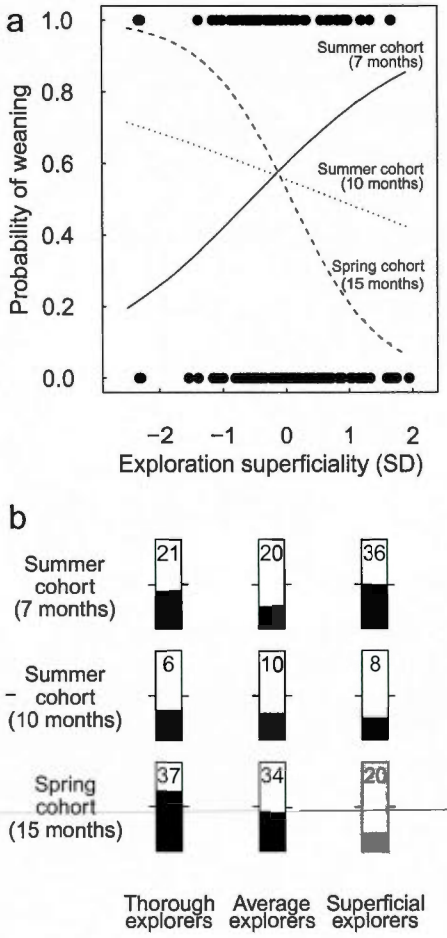

Figure 4.4 a) Probability of weaning at least one juvenile as a function of female exploration and birth cohort (and their age at first breeding opportunity), in the mounts Sutton chipmunk population. Exploration indexes are expressed as standard deviation from the population mean and range from thorough (negative values) to superficial (positive values). Filled dots represent the data available to estimate the interaction (see Table 4.3, 188 female-seasons from 97 females). b) Proportion of thorough, average and superficial exploring females that succeeded in producing at least one juvenile (filled in black) when given a first reproduction opportunity at 7, 10 or 15 months of age. Thorough, average, and superficial exploring females belong to the first, the second and the third thirds of the distribution of exploration patterns, respectively. The number of observations available for each category is displayed in the top of the bars.

Tableau 4.4 Final model analyzing the number of juveniles produced by females during each mating season (N = 97 females, 188 mating seasons, deviance = 482.78. on 180 degrees of freedom, LRT = likelihood ratio test, d.f. = degrees of freedom, the fall season is taken as the reference).

Components	Variance	LRT	d.f.	p
Vi	0.476	12.70	1	<0.001
Vr	0.690			

Terms	Coefficient ± s.e.	t	d.f.	p
Age at first reproduction (7 months)	0.155 ± 0.220	0.70	1	0.480
Age at first reproduction (11 months)	-0.475 ± 0.372	1.27	1	0.200
Age at first reproduction (15 months)	-0.149 ± 0.170	0.87	1	0.380
Exploration (7 months)	0.077 ± 0.250	0.31	1	0.760
Exploration (11 months)	-0.238 ± 0.401	0.59	1	0.550
Exploration (15 months)	-0.735 ± 0.360	2.03	1	0.040
Season	-1.150 ± 0.199	5.76	1	<0.001

Tableau 4.5 Final generalized linear model analyzing male lifetime reproductive success. We included only males known to have produced at least one juvenile in the data set (N = 52, residual deviance = 56.90 on 48 degrees of freedom, d.f. = degrees of freedom).

Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	0.735 ± 0.141	5.21	1	<0.001
Age at first reproduction (15 months)	0.191 ± 0.206	0.92	1	0.360
Exploration pattern	0.238 ± 0.147	1.62	1	0.110
Exploration X age at first reproduction	-0.523 ± 0.210	2.49	1	0.016

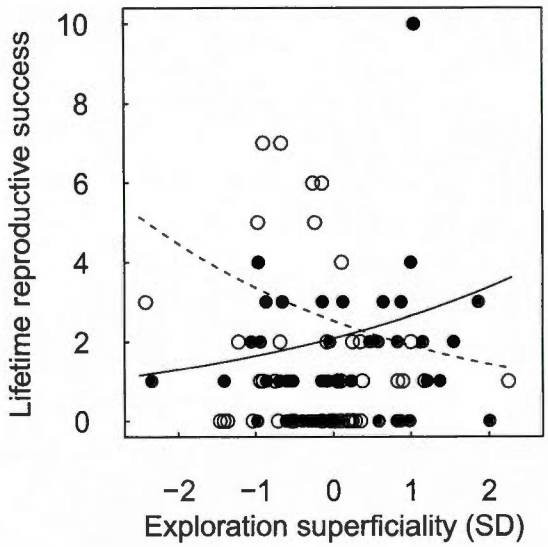

Figure 4.5 Lifetime reproductive success (number of juveniles sired) as a function of male exploration pattern and birth cohort (summer cohort with an early first breeding opportunity : filled dot, solid line, spring birth cohort with a late first breeding opportunity : open dots, dashed line). Exploration indexes are expressed as standard deviation from the population mean and range from thorough (negative values) to superficial (positive values). Omitting the individual with highest reproductive success did not affect the significance of the interaction between exploration and age at first breeding. Estimates were computed from a model including age at first breeding opportunity, exploration pattern and an interaction between exploration pattern and age at first breeding opportunity as fixed effects. Estimates are plotted untransformed.

Tableau 4.6 Linear mixed model analyzing the number of seconds spent active during the bag tests by a) summer born females (N = 25 females, 676 tests), and b) by spring born females (N = 26 females, 1018 tests, LRT = likelihood ratio test, d.f. = degrees of freedom).

a

Components	Variance	LRT	d.f.	p
Vi	0.468	59.69	1	<0.001
Vr	1.208			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	1.714 ± 0.110	15.49	1 (651)	<0.001
Exploration pattern	-0.332 ± 0.100	3.30	1 (23)	0.003

b

Components	Variance	LRT	d.f.	p
Vi	0.681	306.67	1	<0.001
Vr	1.054			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	1.696 ± 0.164	10.33	1 (992)	<0.001
Exploration pattern	-0.308 ± 0.270	1.14	1 (24)	0.270

4.4.4 Relationship between exploration and docility

Superficial explorers were less docile than thorough explorers among females born in the summer (early, intermediate and very late breeders, $F_{1,23} = 10.89$, $p = 0.003$, N = 25 females and 676 bag tests, see Table 4.6a) but not among spring-born females composed of late and very late breeders ($F_{1,24} = 1.30$, $p = 0.26$, 26 females, 1018 bag tests, see Fig. 4.6 and Table 4.6b).

In contrast, exploration pattern was not significantly correlated to docility among

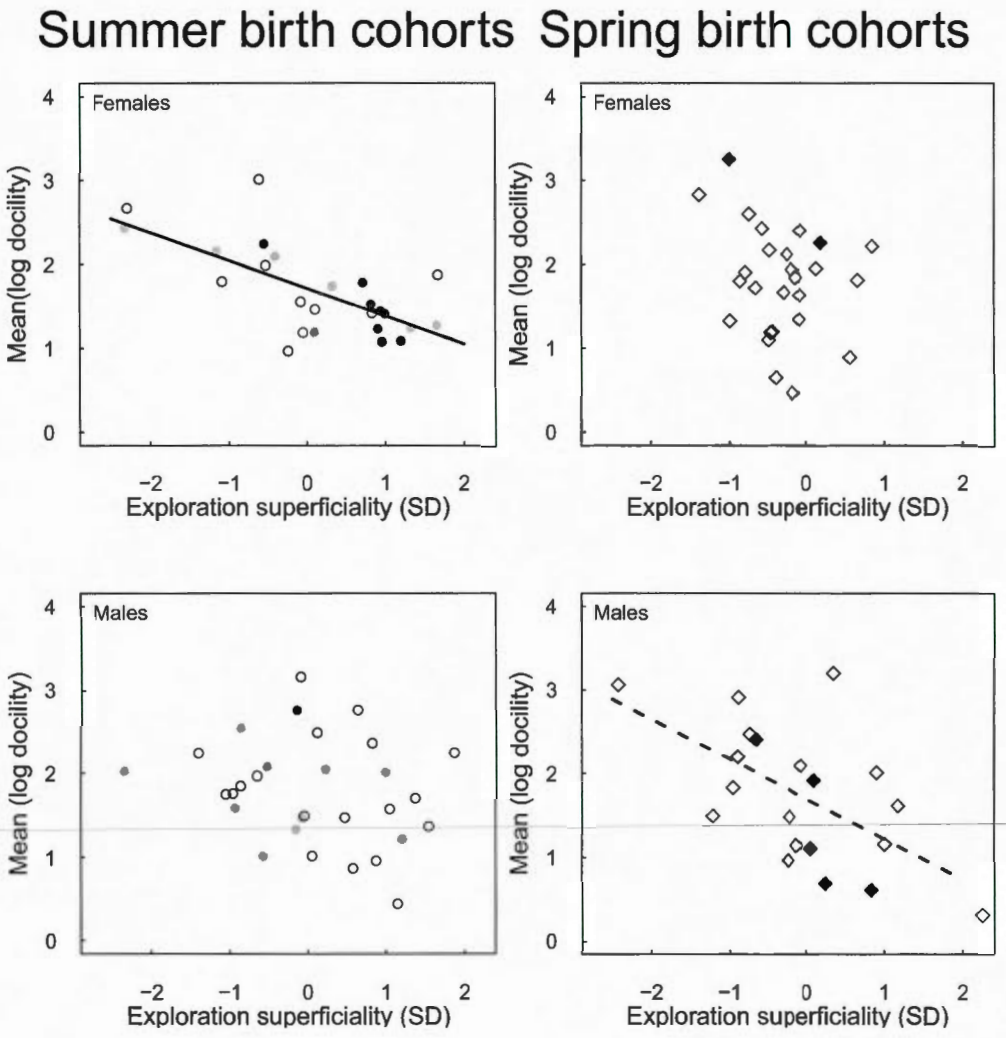

Figure 4.6 Relationship between exploration and the mean number of seconds spent immobile while handled (docility) in females (upper panels) and males (lower panels) from the summer (left panels) and spring (right panels) birth cohorts. Summer-born individuals included early (filled dots), intermediate (gray dots) and very late breeders (open dots, 25 females and 34 males). Spring-born individuals comprised late breeders (filled diamonds) and very late breeders (open diamonds, 26 females and 18 males). Docility was corrected for residual individual differences. Exploration index are expressed as standard deviation from the population mean and range from thorough (negative values) to superficial (positive values). All estimates were computed from a model including only exploration pattern as a fixed effect and individual identity as a random effect.

Tableau 4.7 Linear mixed model analyzing the number of seconds spent active during the bag tests by a) summer born males (N = 34 males, 472 tests), and b) by spring born males (N = 20 males, 454 tests, LRT = likelihood ratio test, d.f. = degrees of freedom).

a

Components	Variance	LRT	d.f.	p
Vi	0.693	101.25	1	<0.001
Vr	1.076			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	1.788 ± 0.134	13.28	1 (438)	<0.001
Exploration pattern	-0.124 ± 0.145	0.85	1 (32)	0.400

b

Components	Variance	LRT	d.f.	p
Vi	0.740	120.47	1	<0.001
Vr	1.043			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept	1.694 ± 0.181	9.33	1 (434)	<0.001
Exploration pattern	-0.477 ± 0.186	2.55	1 (18)	0.020

summer-born males ($F_{1,32}$ = 0.73, p = 0.39, N = 472 bag tests from 34 males, see Table 4.7a), while superficial explorers were less docile among spring-born males ($F_{1,18}$ = 6.53, p = 0.019, N = 454 bag tests from 20 males, see Fig. 4.6 and Table 4.7b).

4.5 Discussion

Our goal was to investigate the relationships between exploration and reproductive life history in an eastern chipmunk population experiencing variable ecological conditions between years. We first investigated the relationship between exploration patterns and age at first reproduction and found that thorough exploring females, but not males

tended to reproduce earlier than more superficial exploring females. Second, we analyzed how the reproductive success of females and males varied as a function of their exploration pattern and age, showing that both females and males with a more thorough exploration pattern expressed a higher reproductive success later in their lives. Third, we tested and found support for the hypothesis that an earlier opportunity to reproduce - associated with summer birth cohorts - increased the reproductive success of more superficial explorers compared to thorough explorers. Finally, we investigated how the strength of a behavioural syndrome between individual exploration pattern and docility varied depending on the birth cohort and the sex of the individuals. In accordance with our prediction, birth cohorts where females displayed higher life history variability showed a stronger relationship between exploration pattern and docility. Surprisingly, we found the opposite pattern for males.

The role of personality traits in life history trade-offs, such as the one between current and future reproduction, is a central assumption of recent adaptive explanations for the maintenance of animal personality in natural populations (Dingemanse et Wolf, 2010; Réale et al., 2010a). Thorough exploring females tended to reproduce later. We also showed that thorough exploring females and males displayed a higher reproductive success later in life. By definition, exploration is associated with the gathering of information incurring immediate costs but future benefits (Hutt, 1969). In chipmunks, exploration may increase the risk of predation or parasitism (Boyer et al., 2010; Patterson et Schulte-Hostedde, 2011). On the other hand, exploration enables chipmunks to sample ephemeral food patches (Hall, Humphries et Kramer, 2007) and is likely to improve spatial knowledge to escape predators (Elliott, 1978) or find mates (Bergeron et al., 2011b). Thus, assuming that exploration patterns during our behavioural assays reflects the exploration in natural settings (Boon, Boutin et Réale, 2008; Boyer et al., 2010), thorough explorers may trade off current reproduction against future reproduction. Such associations may also be state-dependent, and vary with the age, or the sex of the individuals. For example, Quinn et al. (2009) reported complex interactions in how individuals negotiated the trade-offs between risk of starvation, and risk of preda-

tion, their sex, age and exploration behaviour. In the current study, we did not separate females who reproduced but failed to wean any juveniles from the ones who skipped the mating season. Therefore, investigation of the behavioral decisions made by individuals over whether and when to reproduce are beyond the scope of this study. Future studies investigating whether the patterns reported here arise through abandonment of litters or through differing decisions over whether to reproduce in the first place will help clarify these aspects.

In eastern chipmunks, the timing of mating seasons fluctuates from year to year as a function of mast events (Bergeron et al., 2011a). Our results suggest that the timing of such mast events favoured a higher reproductive success of individuals with different exploration patterns, through their effects on age at first opportunity for reproduction. Among spring born individuals, experiencing a later breeding opportunity, more thorough explorers expressed a higher reproductive success. This late breeding opportunity is caused by the dependence of chipmunk mating season on the mast events. A few other studies also reported fluctuating selective pressures on personality traits. In great tits (*Parus major*), for example, selection on individual exploration oscillated between stabilizing and disruptive among years (Dingemanse et de Goede, 2004). Interestingly, great tit population dynamics, like chipmunk populations dynamics, are associated with the occurrence of mast events in some years but not in others. The authors suggested that individual reproductive success depended on the capacity of individuals to acquire food and to defend a suitable territory. Both components are likely to be affected by yearly fluctuations in food abundance, but also by population density (Both, 1998). Similarly, in our studied chipmunk population, years of high food abundance are followed by years of high density because of the increased juvenile recruitment and adult survival (Bergeron et al., 2011a). In this system, fluctuations in population density and food abundance determine the availability of suitable burrows and the amount of reserves individuals may hoard (Bergeron et al., 2011a). It is also possible that density affects individuals differently as a function of their exploration pattern (Both et Visser, 2000). Alternatively, individuals could adjust their behaviour to the environmental conditions

they experience during their first year of life. Further analyses, investigating how individual exploration patterns changes over the life of the individuals as a function of their environment will help disentangle these two processes. These yearly fluctuations in environmental condition associated with food abundance could affect many different aspects of a chipmunk's life history such as dispersal behaviour, above ground activity and mating effort (Bergeron et al., 2011b; Bergeron et al., 2011a). While the patterns we document in this study are interesting, they require a detailed follow up study spanning more years in this population. We also acknowledge that our measurements of reproductive success, particularly in males, may be limiting due to the size of the study area.

We also investigated how the extent of individual variation in life history among birth cohorts predicts the strength of the behavioural syndrome linking exploration pattern and docility. In agreement with our initial prediction, among females born in the summer, including early, intermediate and very late breeders, more superficial explorers were less docile, while we found a much weaker and non-significant association between the two traits in females born in the spring, where most females reproduced at the same age. Surprisingly however, we found the opposite pattern in males, where more superficial explorers were less docile among males born in the spring. In this species, males may face different constraints over their reproductive life-history than females, for example related to intense intrasexual competition for access to mates, or because they display a higher tendency to disperse before reproduction (Chambers et Garant, 2010; Dubuc Messier, Bergeron et Réale, 2012). It is thus possible that fitness expectation related to the age at first reproduction is not a major factor shaping male reproductive life-history in eastern chipmunks. We suggest that differences in the intensity of competition taking place among males in the summer and in the spring may greatly affect male reproductive life-history. Spring-born males experienced their first reproduction mainly during the summer, and thus face an important scramble competition for access to females, which results in high levels of multiple mating (Bergeron et al., 2011b). In contrast, summer-born males experienced their first reproduction in the spring, where competition among

males is presumably lower and essentially characterized by female defence, resulting in lower levels of multiple mating (Bergeron et al., 2011b). In our study, only one male succeeded in reproducing early (i.e. at 7 months of age), suggesting that competition between males, at least during the spring mating season, may prevent younger males from reproducing early.

Our results suggest that personality variation is linked to age-specific reproductive success patterns. However, we did not identify the mechanism generating differences in exploration patterns among individuals with different age at first opportunity for reproduction. Moreover, we did not account for the important variation in factors such as environmental conditions, female reproductive history and physiology. For example, in a previous study in the same population Bergeron et al. (2011c) showed that the physiological costs of reproduction, measured as daily energy expenditure and oxidative damage, were affected by environmental conditions and age. Hence, a re-analysis of exploration patterns in relation to female reproductive history and physiological state may help describe the mechanisms responsible for the variation in exploration patterns.

In conclusion, our study provides support for the current reasoning on the adaptive nature of individual personality. Using a free ranging eastern chipmunk population, we showed that life history variation related to different ecological contexts is associated with consistent individual differences in exploration pattern. Future studies should aim at investigating the behavioural and physiological mechanisms of such relationships.

4.6 Acknowledgements

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CHAPITRE V

PERSONALITY, AGE AND REPRODUCTION AFFECT CORTISOL LEVELS IN EASTERN CHIPMUNKS (*TAMIAS STRIATUS*)

Pierre-Olivier Montiglio, Dany Garant, Fanie Pelletier & Denis Réale

5.1 Abstract

Glucocorticoïds (GC) enable animals to adjust their life history and behaviour to changing environmental conditions and are thus central determinants of their fitness. The relationship between seasonal fluctuations in GC and reproduction is relatively well studied in natural populations, and the behavioural correlates of GC stress reactivity has been the focus of many experimental studies. However, studies have seldom studied simultaneously the relationships between individual behaviour, reproduction and GC levels in natural populations. Fewer studies still have distinguished the seasonal GC fluctuations from individual differences in GC stress reactivity over the long term. 2. Male and female eastern chipmunks (*Tamias striatus*) display contrasting life histories associated with their exploration pattern : more superficial exploring individuals reproduce at an earlier age and display their highest reproductive success early in their life compared to more thorough exploring individuals. Here, we investigated how individual personality, age and reproduction were linked to the faecal cortisol levels and variability in a free ranging population of this species. Males and females of known age and personality were monitored during the breeding season to assess their reproductive status. Faeces from marked chipmunks were also sampled during trapping sessions, over a 5 month period

to assess faecal cortisol levels and daily variability, a proxy of stress reactivity. Faecal cortisol levels were measured by ELISA and analysed using linear mixed models. Female cortisol levels decreased during gestation and lactation compared to non-reproductive females. More thorough exploring females and females with a smaller litter displayed a higher cortisol variability. Male faecal cortisol level during the mating season increased with the number of offspring produced and decreased with age. Overall, our study shows that considering simultaneously the seasonal fluctuations in GC level associated with changes in the energetic state of animals and the dynamic of stress reactivity may help understand how individuals express coordinated suites of life history and behavioural adaptations. Individual differences in stress reactivity are detectable even in natural environmental settings and are likely to have important fitness consequences in wild populations.

5.2 Introduction

Animals have a limited amount of energy they can allocate to different biological functions associated with fitness, leading to evolutionary trade-offs (Stearns, 1992). For example, at any given point in their life cycle, animals must 'choose' to allocate resources to reproduction or survival. In iteroparous species, animals must also make decisions concerning their allocation to current reproduction, versus surviving and reproducing later (Williams, 1966). The balance in allocation between reproductive events or fitness components may vary among species as a function of environmental conditions (Stearns, 1992). For example, species facing high mortality rates because of predation will evolve life history strategies that favour current reproduction at the expense of survival and future reproduction (Stearns, 1992). Such 'fast-living' species will typically have an early age at maturity, a high fecundity but a shorter life span (Gaillard et al., 1989; Bielby et al., 2007). On the other hand, 'slow-living' species, expressing a later age at maturity, a lower fecundity but a longer life span, are often found in environments with low levels of resources (Wiersma, Munoz-Garcia et Williams, 2007). Similar differences are also documented among populations within species (Walsh et Reznick, 2009; Torres Dowdall

et al., 2012), and among individuals within populations (Harris et al., 2010; Krause et Liesenjohann, 2012). Furthermore, recent evidence showed that life history traits are associated with behavioural traits (Wingfield, 2005; Réale et al., 2010a), when the latter are associated with resource acquisition (Biro et Stamps, 2008) or affect mortality risk (Stamps, 2007). Fast-living species or individuals, for example, are predicted to display higher levels of aggressiveness, activity and faster exploration than slow-living species or individuals (Dingemanse et Wolf, 2010; Réale et al., 2010a).

Reproduction is closely associated with the activity of the hypothalamo-pituitary-adrenal (HPA) axis (Reeder et Kramer, 2005). Functioning of the HPA axis culminates by the secretion of glucocorticoïds (GC) into the blood, which have a dual role on both life history and behavioural traits, depending on their concentration (Ricklefs et Wikelski, 2002). Under a given threshold, GC are associated with the organism's energetic state, and increase in response to high energetic demands (Romero, Ramenofsky et Wingfield, 1997; Romero, 2002; Boonstra, 2005), or low food availability (Pravosudov et al., 2001; Romero, Dickens et Cyr, 2009; Kitaysky et al., 2010). At these low levels GC mobilize the resources of the organisms to fuel growth or reproduction (Romero, 2004). GC levels under this threshold also favour the expression of behaviours associated with resource acquisition such as increasing foraging activity or exploration level of animals (Kitaysky et al., 2001; Pravosudov, 2003; Angelier et al., 2007b; Gutman et al., 2011). However, when energetic requirements become too high (Romero, Dickens et Cyr, 2009) or when the animal faces an acute perturbation of its environment (Wingfield, 2005), the HPA axis mounts a brief and intense increase in GC over minutes or hours followed by a rapid decline to lower levels, also called a stress response, (Reeder et Kramer, 2005). GC produced during such short term stress responses shut down reproductive functions and redirect the resources to maximizing survival (Wingfield et Sapolsky, 2003; Reeder et Kramer, 2005; Romero, Dickens et Cyr, 2009) by modifying both the behaviour and the life history of the organism (Wingfield et al., 1994). Therefore, evolutionary trade-offs can be mediated through variation in the threshold over which GC switch from having a positive to a negative effect on current reproduction.

The threshold over which GC stop promoting current reproduction to improve survival or future reproduction varies among species as a function of their life history. For example, species where individuals exhibit low prospects of future reproduction, or display faster life histories, (Kitaysky et al., 1999; Bókony et al., 2009) or species where mating competition is high (Boonstra, 2005) down-regulate their stress reactivity during the reproductive season, thereby avoiding the negative effects of elevated GC for reproduction. Individuals within species may also exhibit variation in this threshold. Individuals investing more in their current reproduction, by producing more numerous or heavier broods, down regulate their stress reactivity more than others during the reproductive season (Lendvai et Chastel, 2008; Bókony et al., 2009). Likewise, older individuals reduce stress reactivity when age is associated with reduced chances of future reproduction (Otte et al., 2005; Heidinger, Nisbet et Ketterson, 2006; Heidinger, Nisbet et Ketterson, 2008).

Investigating both the fluctuations in GC levels, reflecting how much energy is invested in current reproduction, and stress reactivity to environmental perturbations, reflecting how much survival and future reproduction are important for individuals, in an integrated manner would provide insights on how individuals negotiate evolutionary trade-offs (Ricklefs et Wikelski, 2002). Yet these two components of the HPA axis activity are usually studied in isolation. Moreover, current studies often fail to link stress reactivity to direct measures of fitness (Breuner, Patterson et Hahn, 2008) and typically neglect the tremendous variation in GC levels found among individuals from the same population (Williams, 2008). Nevertheless, individuals are known to display consistent differences in their GC levels and in their stress reactivity (Cook et al., 2011; Cook et al., 2012; Smith et al., 2012) and these differences are often studied in relation with animal personality (Koolhaas et al., 1999; Carere et al., 2005; Overli et al., 2007). In such cases, animals that are more exploratory, more active, or more aggressive usually display a consistently higher GC level but a lower GC stress response (Koolhaas et al., 1999; Carere et al., 2005; Overli et al., 2007; Atwell et al., 2012). Individual differences in stress response are mostly investigated in controlled settings (Carere et al., 2003; Fu-

cikova et al., 2009; Baugh et al., 2012), but recent work suggest that they are also detectable over extended periods of time in the wild (Montiglio et al., 2012a). Interestingly, individual differences in stress reactivity and baseline GC are also associated with life history tactics weighting differently current reproduction versus survival and future reproduction (Coppens, de Boer et Koolhaas, 2010; Houston, 2010; Dingemanse et Wolf, 2010; Réale et al., 2010a). An analysis of individual variation in GC level and stress reactivity within populations and in natural settings is therefore needed to investigate how the HPA axis integrates life history and behavioural traits into tactics (McGlothlin et Ketterson, 2008; Williams, 2008).

In this study, we assess the joint effects of individual personality, reproduction and age on both the faecal GC level and variability in a wild population of eastern chipmunks in Southern Québec, Canada. In this species, females provide maternal care to juveniles during their first month of life. Males, in contrast, do not provide any care but face an intense competition to access females during their oestrus and invest heavily in mate searching (Elliott, 1978; Bergeron et al., 2011b). Furthermore, individuals from this population differ consistently in their exploration pattern in a novel environment throughout their entire life, ranging from thorough to superficial (Montiglio et al., 2010). More thorough explorers display a slower life history, characterized by a later age at first reproduction and a higher reproductive success toward the end of life compared to more superficial explorers (see chapter III). Thorough explorers also seem to display a higher faecal cortisol variability over the course of their yearly active season (Montiglio et al., 2012a). We expected that faecal cortisol levels during reproduction would increase with the number of juveniles produced. Such an increase would be observed during gestation and lactation for females, and during the mating season for males. We made the assumption that individuals with a higher reproductive output would favour their current rather than their future reproduction. We thus expected that these individuals would show a lower faecal cortisol variability. Older individuals, having reduced chances of future reproduction should also display reduced cortisol variability compared to younger ones. Finally, along our results from previous studies, we also predicted that thorough

explorers with a slower life history would display a higher faecal cortisol variability, even after accounting for reproduction.

5.3 Materials and methods

5.3.1 Study system and study site

Eastern chipmunks are Sciurids feeding on seeds from masting trees (Landry-Cuerrier et al., 2008) that they hoard in their burrow for the winter (Snyder, 1982). In Québec, chipmunks usually display two mating seasons : a first one occurs in June while a second mating season occurs in March (Bergeron et al., 2011a). Mating seasons last from three to four weeks. During this period, males increase considerably their space use and visit females on their home range prior to their oestrus (Elliott, 1978). A female's oestrus lasts for a day during which there is important scramble competition between males for the access to and the fertilization of females (Elliott, 1978). After about four weeks of gestation, females give birth to two to eight pups that spend the first four to five weeks of their life in the maternal burrow before emerging above ground and dispersing (Elliott, 1978; Snyder, 1982).

We followed a population of chipmunks located on a 25 ha grid in southern Québec (45°05' N, 72°25' E), Canada, from 2005 to 2009. The study site was characterized by a mixed forest dominated by red and sugar maples (*Acer rubrum*, *Acer saccharum*) and American beech (*Fagus grandifolia*). Although they feed on maple seeds and other food sources, chipmunks rely mostly on beech tree seeds for their reproduction and above ground activity. Their life cycle is strongly linked to the contrasted inter-annual fluctuation in beech seed production (Bergeron et al., 2011a). Every year between 2006 and 2010, from May to October, we trapped chipmunks using Longworth traps. Traps were baited with peanut butter, opened at $\sim$ 0800 in the morning, inspected every two hours and closed at dusk. Upon capture, individuals were systematically marked with metal ear tags and a passive integrated transponder. We also sexed, weighed and sampled tissues from each individual for DNA analyses (see next section).

5.3.2 Reproduction

Adult individuals were equipped for a short period ($\sim$ 1 week) with radio transmitters to locate their burrow ($\sim$ 85 % of the individuals). We carried a daily trapping routine of females between the mating season and juvenile emergence to determine the reproductive status (i.e. pre-reproductive, gestating, lactating or post-reproductive) of females based on the nipple and vulva aspect (Bergeron et al., 2011a). Females showing no sign of reproductive activity or pregnancy where considered as 'non-reproductive' for the entire season and assigned a litter size of 0. We captured the juveniles directly at the mother's burrow before their dispersal to estimate litter size at weaning (Bergeron et al., 2011b). We considered the number of offspring that emerged from the burrow (i.e. referred to as number of young produced thereafter) as an index of a female reproductive success at each season. Males were all considered as reproductively active for a period of six weeks before the peak of oestrus in the population (referred to as the 'mating season'), which corresponds to the period where they expand their home range (Montiglio, unpublished). We confirmed maternal identity and determined the paternity of the juveniles each year using 11 microsatellite loci (Chambers et Garant, 2010; Bergeron et al., 2011b) and the software CERVUS 3.0.3 (Kalinowski, Taper et Marshall, 2007) using a 95 % confidence level. We considered all males trapped on the study site during a year as potential fathers. Note that because males have wide home ranges during the reproductive season, we were unable to determine the absolute number of young produced by each male (Bergeron et al., 2011b). We considered the number of offspring attributed to a male as an index of male reproductive success at each season (referred to as number of young produced, thereafter).

5.3.3 Faecal cortisol collection and assay

We collected faecal samples from the traps during each capture in 2009. Faecal samples contaminated by urine were discarded. The samples were immediately transferred to test tubes and kept on ice before being transferred to a - 20 °C at the end of the

day. The samples were then stored in a - 80 °C freezer within two weeks of collection. The faecal samples were first dried to constant mass in an oven (70 °C) and pulverised using a glass plunger. We vortexed ~ 35 mg of faecal matter in methanol (80 %) for 20 minutes (15 000 rpm), centrifuged the liquid and collected the supernatant for an enzyme immunoassay using a cortisol antibody with a horseradish peroxidase ligand R4866, from Coralie Munro, California, Riverside (Young et al., 2004). Samples were analysed in duplicates and we re-analysed all samples showing a coefficient of variation higher than 20 %. Intra- and inter-assay coefficients of variation were 8.42 % and 9.86 % respectively. We previously showed that this assay detects a cortisol response to a standardized ACTH challenge test, as well as circadian variations in faecal cortisol levels in eastern chipmunks (Montiglio et al., 2012a) and that faecal samples collected in this species represent an integrated measure of the cortisol produced over a period of ~ 8 h preceding defecation (Montiglio et al., 2012b).

5.3.4 Behavioural assays

Chipmunks were tested in the open-field test twice during their lifetime between 2006 and 2010. Prior to the test, we transferred the chipmunks from the trap to a handling bag and identified it without any manipulation with a hand held transponder reader. The individual was then transferred to a small chamber connected to the arena. We introduced the animal into the arena by lifting a small door connecting the chamber and the arena and videotaped its behaviour for 90 seconds. We quantified chipmunk behaviour in the open-field with the software The Observer 5.0 (Noldus Technology 2003). Lines were drawn on the floor of the arena and we measured exploration as the number of lines crossed during the three consecutive 30-seconds intervals. Coding the behaviour of chipmunks by intervals enabled us to determine the temporal patterns of exploration within the tests. Individuals expressed patterns ranging from thorough, characterized by an intermediate but constant exploration level throughout the test, to superficial, characterized by a high exploration level at the beginning of the test followed by a steep decline over the following seconds. The activity pattern in the

open-field displays a repeatability of $\sim$ 30 %. We built a model accounting for the effects of date, age, sex, year, and trial order on exploration levels. The model also included individual identity as a random effect. We used the Best Unbiased Linear predictors (BLUPs) from this model as individual indexes of exploration for the rest of the analysis. The exploration index represents the deviation of each individual from the population's predicted mean exploration pattern in standard deviation units (Pinheiro et Bates, 2000). Additional details about the test and exploration index can be found in Montiglio *et al.* (2010; 2012b).

5.3.5 Statistical analyses

We analysed faecal cortisol levels for each sex using linear mixed models. Faecal cortisol level was log transformed to attain normality. We initially included as fixed effects exploration index, age in months, number of young produced, reproductive status (females : non-reproductive, pre-reproductive, gestating, lactating or post-reproductive; males : reproductively-active during the mating season or not reproductively active after the mating season) and all their two ways interactions in the model. Individual identity and day of sampling (nested within the individual) were fitted as categorical random effects to account for pseudo-replication and daily variability (Pinheiro et Bates, 2000). In addition, we investigated how individual reproduction, personality and age affected individual daily variability by fitting an effect of the number of juveniles produced, the exploration pattern and the age on the residual variance. To investigate whether the relationship between reproduction and cortisol variability vary with the exploration pattern of the individuals, we also tested for an interaction between exploration and the number of young produced (Pinheiro et Bates, 2000). We first simplified the fixed effect structure of each model in a backward - forward stepwise manner using Aikake's information criterion (Burnham et Anderson, 2002). We then tested for the significance of the variance covariates using likelihood ratio tests (Pinheiro et Bates, 2000). This test uses one degree of freedom and compares the ratio between the log-likelihood of the model with the variance covariate of interest and a model without it. Preliminary

analyses showed that a temporal autocorrelation structure did not significantly improve the models (results not shown). All linear mixed models were fitted in R 2.14.1 using the package nlme (Pinheiro et Bates, 2000). We report means and estimates ± SE.

5.4 Results

5.4.1 Females

We collected 287 faecal cortisol samples from 30 females (9.57 samples/females, range = 2 - 26). Model selection by AIC yielded one single best model, with an AIC lower by 2 units or more than the other models (see Table 5.2). Cortisol level was lower in lactating females compared to non-reproductive ones ($F_{4,25} = 4.131$, $p = 0.010$, see Fig. 5.1 and Table 5.2 for all the estimates). Faecal cortisol level increased as a function of age ($F_{1,27} = 13.66$, $p = 0.001$, see Fig. 5.2). Exploration level was included in the final model, but failed to reach significance ($F_{1,27} = 2.09$, $p = 0.160$).

Individual daily variability in faecal cortisol decreased with the superficiality of female exploration pattern (LRT = 6.23, df = 1, p = 0.012 see Fig. 5.3 and Table 5.2) and with the number of young they produced (LRT = 6.89, df = 1, p = 0.009, see Fig. 5.4). Female age (LRT = 0.04, df = 1, p = 0.84) and the interaction between exploration and number of young produced (LRT = 2.07, df = 1, p = 0.15) had no effect on within-group variance in faecal cortisol.

5.4.2 Males

We collected 161 faecal cortisol samples from 27 males (5.96 samples/males, range = 1 - 19). Model selection using AIC yielded two models with close AICs (see Table 5.3). The two models differed by only one term, with a negligible effect size (an interaction between age and siring success, with an estimate of 0.009 ± 0.001). Since all the other estimates where almost identical in both models, we chose to present the most parsimonious model. Contrary to females, male faecal cortisol level was higher in reproductively active

Tableau 5.1 AIC model simplification for faecal cortisol level and daily variability in female eastern chipmunks in 2009 (287 samples from 30 females). AIC was used to simplify the fixed effect structure only.

Variables	K	AIC
age + reproductive status + exploration + litter size + age x reproductive status + age x exploration + age x litter size + reproductive status x exploration + reproductive status x litter size + exploration x litter size	22	857.88
age + reproductive status + exploration + litter size + age x reproductive status + age x exploration + age x litter size + reproductive status x litter size + exploration x litter size	18	850.53
age + reproductive status + exploration + litter size + age x reproductive status + age x exploration + age x litter size + reproductive status x litter size	17	848.54
age + reproductive status + exploration + litter size + age x reproductive status + age x litter size + reproductive status x litter size	16	846.91
age + reproductive status + exploration + litter size + age x reproductive status + age x litter size	12	846.45
age + reproductive status + exploration + litter size + age x litter size	8	843.79
age + reproductive status + exploration + litter size	7	841.79
age + reproductive status + exploration	6	839.88

Tableau 5.2 Final model analysing faecal cortisol level and variability in female eastern chipmunks in 2009 (287 samples from 30 females). Significance of the variance covariates and random effects was tested using likelihood ratio tests (LRT, see 'Statistical analyses' section). Faecal cortisol was log-transformed prior to analysis.

Components	Variance	LRT	d.f.	p
Vi	0.215	25.70	1	<0.001
Vd	0.814	15.43	2	<0.001
Vr	0.778			

Variance covariates	Estimate	LRT	d.f.	p
Exploration	-0.268	6.23	1	0.012
Litter size at weaning	-0.261	6.89	1	0.009

Terms	Coefficient ± s.e.	t	d.f.	p
Reprod. status (non-reproductive)	10.186 ± 0.220	46.25	1 (225)	<0.001
Reprod. status (pre-reproductive)	10.42 ± 0.165	63.09	1 (28)	<0.001
Reprod. status (gestating)	9.858 ± 0.261	37.73	1 (28)	<0.001
Reprod. status (lactating)	9.677 ± 0.116	82.79	1 (225)	<0.001
Reprod. status (post-reproductive)	9.961 ± 0.132	75.11	1 (225)	<0.001
Age	0.027 ± 0.007	3.69	1 (28)	0.001
Exploration	0.125 ± 0.086	1.44	1 (28)	0.159

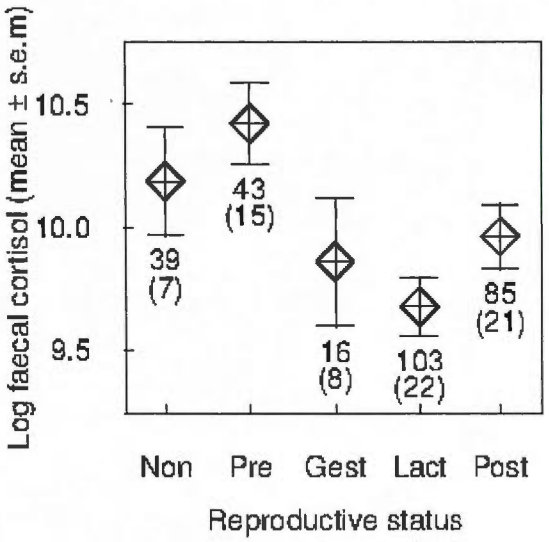

Figure 5.1 Female faecal cortisol level as a function of reproductive status (NON = non-reproductive, PRE = pre-reproductive stage, GEST = gestating stage, LACT = lactating stage, POST = post-reproduction stage). Number of samples available for each reproductive status class is given below the estimates, along with the number of individuals sampled, in parentheses (N = 287 samples from 30 females).

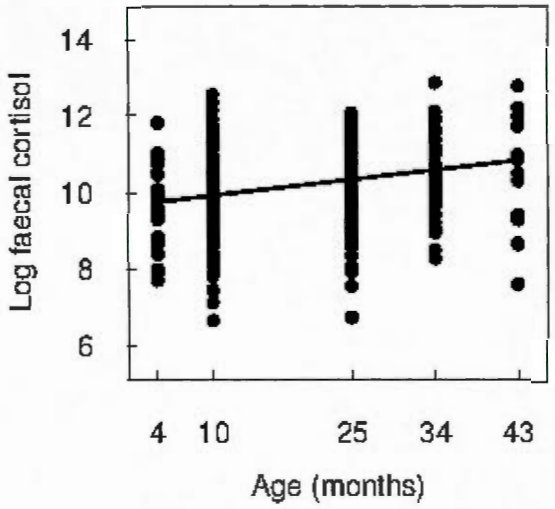

Figure 5.2 Female faecal cortisol level as a function of age (in months). The line represents the estimated age effect presented in Table 5.2 (N = 287 samples from 30 females).

individuals ($F_{1,131}$ = 19.06, p <0.001, see Table 5.4 for all model estimates), but less so in older individuals (age main effect : $F_{1,22}$ = 0.90, p = 0.351; interaction between reproductive status and age : $F_{1,131}$ = 4.68, p = 0.032, see Fig. 5.5). Although they failed to reach significance, siring success ($F_{1,22}$ = 3.54, p = 0.073), exploration pattern ($F_{1,22}$ = 2.08, p = 0.162), as well as the interactions between age and exploration patterns ($F_{1,22}$ = 2.34, p = 0.140) and between reproductive status and siring success ($F_{1,131}$ = 1.99, p = 0.160) were kept in the model yielding the best AIC (see Table 5.3).

Exploration pattern, siring success and age had no effect on individual daily variability (all rejected with p >0.4). Individual identity and day of sampling (random effects) did not explain any significant proportion of daily variability in cortisol in males (all p >0.6).

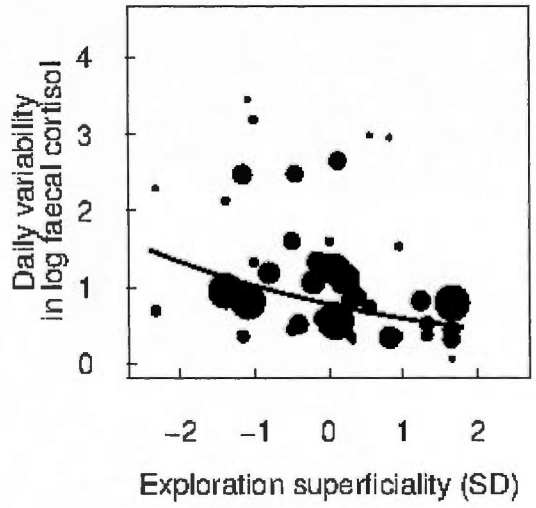

Figure 5.3 Relation between female exploration pattern and variability in log faecal cortisol. Dots represent the observed variance in faecal cortisol of each female and the lines represent the estimated variance function parameter from the model presented in Table 5.2 (N = 287 samples from 30 females). The size of each point is proportional to the number of samples available for each individual.

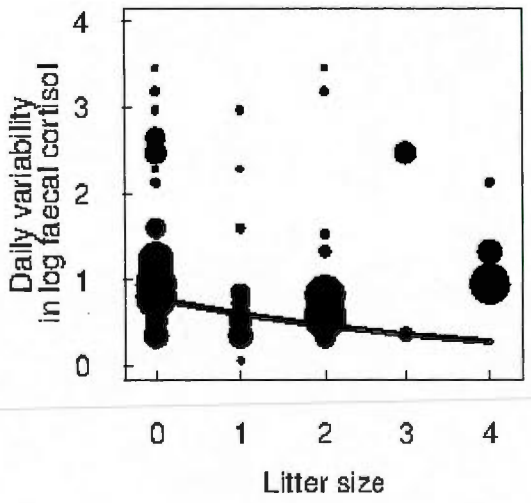

Figure 5.4 Relation between female litter size and variability in log faecal cortisol. Dots represent the observed variance in faecal cortisol of each female and the lines represent the estimated variance function parameter from the model presented in Table 5.2 (N = 287 samples from 30 females). The size of each point is proportional to the number of samples available for each individual.

Tableau 5.3 AIC model simplification for faecal cortisol level and daily variability in male eastern chipmunks in 2009 (161 samples from 27 males). AIC was used to simplify the fixed effect structure only.

Variables	K	AIC
exploration + reproductive status + age + siring success + reproductive status x exploration + age x exploration + siring success x exploration + age x reproductive status + siring success x reproductive status + age x siring success	11	462.34
exploration + reproductive status + age + siring success + exploration x age + exploration x siring success + reproductive status x age + reproductive status x siring success + age x siring success	10	460.34
reproductive status + age + siring success + exploration x age + reproductive status x age + reproductive status x siring success + age x siring success	9	458.45
reproductive status + age + siring success + exploration x age + reproductive status x age + reproductive status x siring success	8	457.28

Tableau 5.4 Final model analysing faecal cortisol level and daily variability in male eastern chipmunks in 2009 (161 samples from 27 males). Significance of the variance components and covariates was tested using likelihood ratio tests (LRT, see 'Statistical analyses' section). Faecal cortisol was log-transformed prior to analysis.

Components	Variance	LRT	d.f.	p
Vi	0.001	0.001	1	1.000
Vr	0.944			
Terms	Coefficient ± s.e.	t	d.f.	p
Intercept (reproductive)	10.352 ± 0.186	55.46	1 (131)	<0.001
Exploration	0.151 ± 0.104	1.44	1 (22)	0.162
Reprod. status (non-reproductive)	-0.959 ± 0.219	-4.36	1 (131)	<0.001
Age	-0.027 ± 0.014	-1.83	1 (22)	0.079
Reprod. success	0.255 ± 0.129	1.96	1 (22)	0.062
Exploration X age	0.012 ± 0.009	1.52	1 (22)	0.140
Reprod. status X age	0.035 ± 0.016	2.16	1 (131)	0.032
Reprod. status X reprod. success	-0.217 ± 0.154	-1.41	1 (131)	0.160

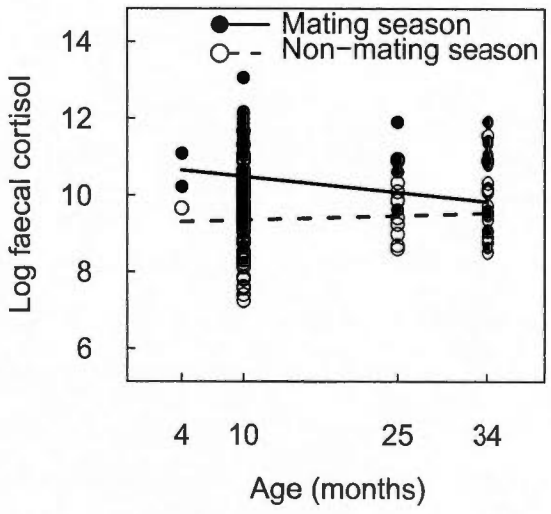

Figure 5.5 Faecal cortisol levels in reproductive (black dots, solid line) and non-reproductive male eastern chipmunks (empty dots, dashed line) as a function of age. Lines represent the estimates presented in Table 5.4 (N = 161 samples from 27 males).

5.5 Discussion

In this study, we quantified the contributions of animal personality and reproduction on individual GC levels and variability in a natural, uncontrolled environment. Our predictions were based on the assumption that higher stress reactivity (i.e. thorough exploration patterns) should translate into higher cortisol variability in natural environments (Montiglio et al., 2012a). Our hypothesis was confirmed by the negative relationship between the superficiality of exploration in an open-field test and daily cortisol variability in females. Contrary to our predictions, we found that females displayed reduced faecal cortisol levels during gestation and lactation. Male faecal cortisol levels varied during the mating season as a function of their age and the number of juveniles they sired. In addition, we also found that females producing larger litters displayed lower cortisol variability. Surprisingly, we found no such pattern in males.

5.5.1 Average faecal cortisol levels

Mean levels of faecal cortisol are thought to fluctuate in relation to the energetic state of individuals, increasing when food availability is low or when energy expenditure is high (Romero, 2004). In rodents, energy expenditure is typically higher during lactation than during any other phase of the life cycle (Naya et al., 2008). In a closely related chipmunk species (*Tamias amoenus*), females also display higher plasma cortisol levels during lactation, potentially reflecting a higher baseline level (Kenagy et Place, 2000). In contrast, we showed that female eastern chipmunks displayed lower faecal cortisol levels during gestation and lactation. A similar pattern has recently been reported for lactating marmots (Smith et al., 2012), where females displayed lower faecal cortisol levels during lactation. One explanation for such a discrepancy in the results is that female chipmunks decrease their activity substantially during gestation and lactation (Montiglio et al. unpublished), and activity is usually associated with high energy expenditure (Astheimer, Buttemer et Wingfield, 1992; Humphries, Thomas et Kramer, 2001; Fletcher et al., 2012). We also found that mean faecal cortisol levels increased with female age. Previous studies also documented heightened mean GC levels in older or more experienced individuals during the reproductive season, which is thought to be associated with the higher reproductive output of such individuals (Angelier et al., 2006). In our analyses, however, litter size failed to explain female cortisol level. Future investigations of the energy requirements and of the behaviour of individuals as a function of their age will surely provide additional insights on this pattern.

Studies of stress reactivity typically use an experimental approach, measuring the response of individuals to a single standardized perturbation (Wingfield, 2005). Our study stands apart from such analyses in that it follows stress reactivity indirectly and in response to the natural environment experienced by animals. Using this rather new approach, we found that females with a more thorough exploration pattern displayed a higher variability in fecal cortisol in the wild. Consistent individual differences in stress reactivity have been documented recently in other systems using a more typical protocol

(Cook et al., 2012). Likewise, individuals with higher stress reactivity are usually less active during open-field tests, shyer, and less aggressive in laboratory settings (Koolhaas et al., 1999; Carere et al., 2005; Overli et al., 2007). However, it is still rare to document such consistent individual differences in stress reactivity in natural settings and over a longer period of time than those typically associated with laboratory studies. More interestingly, exploration pattern is associated with reproductive life history in this population (Montiglio et al. unpublished). The patterns we report here for females are thus in accordance with the idea that the cortisol produced during the stress response favors future reproduction and survival at the expense of current reproduction (Boonstra, 2005; Reeder et Kramer, 2005). It is also important to note that, in free ranging animals, low within-individual variability in cortisol may reflect a lower exposure to environmental perturbations. As such, females with bigger litter sizes or thorough exploration patterns (having lower fecal cortisol variability) may decrease the amount of energy involved in stress responses by avoiding riskier or less predictable habitats. Further studies on this population should thus focus on investigating the behavior and habitat use of individuals, as a function of their reproduction and exploration pattern.

5.5.2 Daily faecal cortisol variability and stress reactivity

In addition to investigating the average faecal cortisol levels, we also assessed faecal cortisol variability. In accordance with our predictions, we found that females with larger litters displayed reduced faecal cortisol variability. Assuming that the variability reported here is mostly affected by individual stress reactivity, females with larger litters would be maximizing their current reproduction by limiting the amount of energy invested in survival functions (Stearns, 1992). Down regulation of stress reactivity is associated with a higher reproductive performance in other species as well. For example, black-legged kittiwakes (*Rissa tridactyla*) display reduced stress responses when submitted to a standardized blood sampling challenge during the egg-laying phase, which would increase their fecundity (Kitaysky, Wingfield et Piatt, 1999; Kitaysky et al., 1999; Kitaysky et al., 2010). In the same species, birds in better condition were even

able to show a stronger decrease in stress reactivity (Kitaysky et al., 1999). Conversely, increased stress reactivity has been associated with lower reproductive success or increased survival in other species (Jessop, 2001; Breuner, Patterson et Hahn, 2008). For instance, high variability in faecal GC is correlated with reproductive cycle disruption in captive black (*Diceros bicornis*), and white (*Ceratotherium simum*) female rhinoceroses (Carlstead et Brown, 2005). The stress response induces the degradation of protein and glycogen stores (Sapolsky, Romero et Munck, 2000) and higher stress reactivity is associated with lower fitness in conditions of starvation in some systems (Romero et Wikelski, 2001; Romero et Wikelski, 2010). Thus, stress reactivity may reduce reproductive success also by increasing energy expenditure, associated with energy conversion. In chipmunks, food availability fluctuates dramatically from one year to the next and strongly shapes the reproduction, survival and activity of individuals in this population (Munro, Thomas et Humphries, 2008; Bergeron et al., 2011a). Hence down regulating stress reactivity, by lowering energy expenditure is likely to have an important effect on individual fitness in this system. Clearly, more studies investigating the cortisol level and variability of individuals across years with differing food abundance will provide more insights on the importance of these processes. Interestingly, we still detected consistent differences in cortisol levels among individuals, even after accounting for their exploration pattern, reproduction and age. Such consistent differences suggest that additional factors, not measured in this study, are likely to affect cortisol differences among females.

We also found that females with a more thorough exploration pattern displayed a higher variability in faecal cortisol. Consistent individual differences in stress reactivity have been documented recently in other systems (Cook et al., 2012). Individuals with higher stress reactivity are usually less active during open-field tests, shyer, and less aggressive in laboratory settings (Koolhaas et al., 1999; Carere et al., 2005; Overli et al., 2007). However, it is still rare to document such consistent individual differences in stress reactivity in natural settings and over a longer period of time than those typically associated with laboratory studies. Interestingly, exploration pattern is associated

with reproductive life history in this population (Montiglio et al. unpublished). The patterns we report here for females are thus in accordance with the idea that the cortisol produced during the stress response favours future reproduction and survival at the expense of current reproduction (Boonstra, 2005; Reeder et Kramer, 2005). It is also important to note that, in free ranging animals, low within-individual variability in cortisol may reflect a lower exposition to environmental perturbations. As such, females with bigger litter sizes or thorough exploration patterns (having lower faecal cortisol variability) may decrease the amount of energy involved in stress responses by avoiding riskier or less predictable habitats. Further studies on this population should thus focus on investigating the behaviour and habitat use of individuals, as a function of their reproduction and exploration pattern.

Surprisingly, exploration pattern and reproductive effort did not seem to affect daily variability in faecal cortisol levels of males. Males also failed to show any significant individual differences in cortisol levels. We acknowledge that the lower sample size available for males, in addition to the difficulty of estimating their absolute reproductive effort, may have limited our capacity to detect these effects in males. However, it has been suggested that males in many squirrel species could either maximize their reproductive success through mate searching or mate guarding (Koford, 1982; Koprowski, 1993). Bigger or more aggressive males may thus invest more in mate guarding (Schulte-Hostedde, Millar et Gibbs, 2002). In our study, older males expressed lower mean faecal cortisol levels compared to younger ones during the mating season (see previous section). Mate guarding may be associated with lower energy expenditure, but further studies, quantifying the energetic costs of male mating tactics in eastern chipmunks are necessary to shed light on the lack of pattern we report here.

In conclusion, our study, by documenting the relationships between faecal cortisol, reproduction, age and personality in a free ranging chipmunk population, suggests that quantifying both the fluctuations in the mean levels of faecal cortisol and its variability may provide important insights on how animals regulate their reproduction and integrate their behaviour and life history. Future studies should complement this approach

by investigating how environmental fluctuations, such as in food abundance, interact with the endocrinology of females and males over extended periods in the wild.

5.6 Acknowledgements

We thank Nature Conservancy of Canada and Ruiter Valley Land Trust for access to study site. We also thank Hélène Presseault-Gauvin for valuable assistance during the faecal cortisol assays and all graduate students and research assistants that have contributed to the data collection. This work was funded by Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery grants (DG, DR, and FP) and Canada Research Chairs program (CRC; DR and FP) and by a team research project grant from the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT; DR, DG, Murray Humphries, Don Kramer and the late Don Thomas). POM was supported by a scholarship from FQRNT. All manipulations, captures and tests were conducted following the guidelines of the Canadian Council on Animal Care via the Université du Québec at Montréal (permit numbers CIPA 0603-462-0607 and 0507-613-0509). The authors declare no conflict of interest.

CHAPITRE VI

CONCLUSION

Un des principaux objectifs de l'écologie évolutive est de comprendre la diversité des traits montrés par les organismes en précisant les implications de ces traits pour leur écologie et leur aptitude phénotypique. L'objectif général de cette thèse s'insert dans donc dans ce cadre, en analysant les relations entre le patron d'exploration des individus, leur reproduction, et le niveau de cortisol régulant à la fois le comportement et la reproduction des individus.

6.1 Contributions

J'ai tout d'abord présenté une étude validant une méthode permettant de mesurer le niveau de métabolites du cortisol présents dans les fèces chez le tamia rayé. J'ai démontré que le niveau de ces métabolites de cortisol reflète la quantité de cortisol produite et sécrétée dans le sang de l'animal durant les 8 dernières heures, et que ces mesures sont aptes à détecter un test d'ACTH. Une manipulation de routine des animaux, similaire à celle qu'ils subissent lors des captures sur l'aire d'étude, a également entraîné une augmentation du niveau de métabolites de cortisol, mais cette augmentation n'a pas atteint le seuil de signification statistique. Dans un deuxième temps, j'ai documenté les relations entre le patron d'exploration exprimé par les individus lors de tests d'arène, leur docilité lors des manipulations sur le terrain et la propension des individus à être capturés dans les pièges. Les individus exprimant un patron d'exploration plus superficiel dans l'arène sont également moins dociles. Ils sont aussi capturés plus fréquemment

(dans le cas des mâles) ou plus loin de leur terrier (dans le cas des femelles). Les individus exprimant un patron d'exploration superficiel dans l'arène démontrent une réactivité du système sympathique plus importante. À l'inverse, l'étude de la variabilité en cortisol des individus au cours de cinq mois suggère que ces mêmes individus mobilisent moins de cortisol en réponse aux perturbations de leur environnement et protègent moins leur survie et leur reproduction future. J'ai dans un troisième temps montré que les individus plus superficiels dans leur exploration ont tendance à se reproduire plus tôt (dans le cas des femelles) et à atteindre leur succès reproducteur maximal plus tôt au cours de leur vie reproductive que les individus plus méticuleux (dans le cas des deux sexes). Ces résultats sont en accord avec mes prédictions (voir la section 'Prédictions' dans l'introduction). Les variations d'abondance nourriture d'une année à l'autre, à travers leurs effets sur l'âge à la première reproduction des individus, contribuent à favoriser un meilleur succès reproducteur à vie des explorateurs superficiels, chez les individus nés durant les années de forte abondance de nourriture, ou encore des explorateurs plus méticuleux, chez les individus nés durant les années de faible abondance de nourriture. L'étroitesse de la relation entre le patron d'exploration et la docilité varie selon le sexe et la cohorte de naissance. Chez les femelles, cette relation est plus étroite lorsque les individus expriment de plus fortes différences au niveau de leur âge à la première reproduction. J'ai par contre observé une relation inverse chez les mâles, allant à l'inverse de mes prédictions. Enfin, j'ai analysé les relations entre le niveau de cortisol, la réactivité au stress, le succès reproducteur et le patron d'exploration des individus. Les femelles ayant un patron d'exploration plus superficiel montrent une variabilité plus faible de leur niveau de cortisol, suggérant qu'elles produisent moins de cortisol en réponse aux perturbations environnementales. Les femelles amenant un plus grand nombre de jeunes au sevrage ont également une variabilité en cortisol plus faible au cours de l'été. La réponse de cortisol aux perturbations environnementales redirige les ressources de l'organisme vers les fonctions maximisant la survie et la reproduction future, au détriment de la reproduction immédiate. Ces résultats sont donc cohérents avec les analyses précédentes, démontrant un plus grand succès reproducteur en début de vie reproductive chez les femelles exprimant un patron d'exploration plus superficiel. Les effets indépendants de

la personnalité et de la reproduction sur le niveau de cortisol suggèrent également que, non seulement les différences physiologiques associées à la personnalité des individus pourraient déterminer leur vie reproductive mais également que leur patron de reproduction serait en mesure d'affecter leur comportement. Ceci peut avoir des implications importantes sur le plan évolutif, en modifiant comment les traits de personnalité et biodémographiques répondent aux pressions de sélection de l'environnement (McGlothlin et Ketterson, 2008). Cette thèse, en utilisant un suivi à long terme et relativement détaillé sur le comportement, la reproduction et la physiologie des individus d'une population naturelle contribue donc à notre compréhension des mécanismes maintenant les différences individuelles de train de vie. Les études empiriques de ces relations en nature sont encore rares. De plus, ces analyses se distinguent des travaux précédents sur le patron de cortisol par le fait qu'elles suivent de manière indirecte, mais à long terme et dans un environnement naturel, la réactivité au stress des individus en fonction de leur personnalité et de leur vie reproductive.

6.2 Analyses futures

La prémisse principale sur laquelle reposent les prédictions formulées durant cette thèse est que le comportement exploratoire est associé à la manière dont les individus négocient le compromis évolutif entre la reproduction précoce et la reproduction en fin de vie reproductive. Plus précisément, le comportement exploratoire impliquerait des coûts en termes de reproduction qui sont immédiats. En revanche, ce comportement générerait des informations sur l'environnement qui seraient bénéfiques au succès reproducteur future. Malheureusement, l'analyse des coûts et des bénéfices associés au comportement exploratoire va au delà de l'objectif de cette thèse. Préciser leur nature augmenterait grandement notre compréhension et notre capacité de prédiction concernant les différences individuelles d'exploration. Par exemple, une étude récente montre que les individus ayant un patron d'exploration extrèmement méticuleux ou extrèmement superficiel ont une survie plus élevée que les individus ayant un patron d'exploration intermédiaire (Bergeron et al., 2013). Ainsi, considérer non seulement

les relations entre le patron d'exploration et le succès reproducteur, mais également ses relations avec la survie des individus de la population contribuer à améliorer notre compréhension des mécanismes maintenant les différences d'exploration dans cette population. Il est également possible que l'exploration implique des dépenses énergétiques, limitant ainsi la reproduction des individus qui expriment ce comportement. L'exploration aurait également des bénéfices pour l'aptitude phénotypique des individus à long terme. En générant une meilleure information sur l'environnement, l'exploration pourrait contribuer à des comportements d'approvisionnement plus efficaces ou permettre de mieux exploiter les refuges contre la prédation (Elliott, 1978). Mieux comprendre la nature des coûts et bénéfices associés au patron d'exploration des individus nécessiterait ainsi de déterminer les relations entre le patron d'exploration d'un individu et ces différentes facettes de son écologie. Par exemple, des suivis télémétriques, permettant de suivre l'utilisation de l'espace, la sélection d'habitat et le comportement de dispersion des individus, ou encore des observations focales analysant le comportement d'approvisionnement des individus permettraient de tester les hypothèses avancées ici et de déterminer de quelle manière les traits de personnalité contribuent aux compromis évolutifs, un aspect central de l'hypothèse des trains de vie.

Enfin, mes analyses ne permettent pas de distinguer les différentes sources de variation contribuant aux différences individuelles d'exploration que j'ai documentées dans la population d'étude. Il est nécessaire de connaître la nature de ces sources de variation pour prédire comment les traits d'histoire de vie et les traits biodémographiques répondraient aux pressions de sélection, et déterminer si les variations de train de vie sont adaptatives. Ces différences d'exploration sont répétables et pourraient donc être associées à une variation génétique entre les individus. On a déjà démontré que les traits de personnalité possèdent des bases génétiques additives dans d'autres systèmes d'étude (Benus, Bohus et van Oortmerssen, 1991; Réale et al., 2000). D'un autre coté, il est possible que ces différences d'exploration soient dues à des effets environnementaux, influençant le phénotype des individus de manière permanente. Ces effets développementaux se produisent souvent avant la naissance, et durant le sevrage (chez les mammifères) à travers

l'environnement maternel (Felszeghy, Bagday et Nyakas, 2000; Seckl, 2001). En particulier, les gluco-corticoïdes produits par la mère durant le développement des jeunes peuvent exercer des effets importants sur le développement et le phénotype des jeunes (Dufty et al., 2002), en influençant leur réponse face au stress, leur comportement et leur biodémographie (Maccari, 1995; Catalani et al., 2000; Jarvis et al., 2006; Love et Williams, 2008b). Ainsi, les rats nés de mères ayant subi des perturbations environnementales quotidiennes durant la grossesse montrent des niveaux d'exploration différents face à un environnement nouveau (Barbazanges et al., 1996; Lordi et al., 2000; Rimondini et al., 2003). Le niveau de GC maternel peut également avoir des effets sur les traits biodémographiques, comme la masse à la naissance, et le taux de croissance (Love et al., 2005). De tels effets maternels pourraient être bénéfiques à la mère, en lui permettant d'ajuster les coûts énergétiques de la reproduction en fonction de leur condition, en régulant le taux de croissance des jeunes durant la période de soins maternels par exemple (Breuner, 2008; Love et Williams, 2008b). Dans le cas du tamia rayé, on peut effectivement s'attendre à ce que les femelles investissent de manière différente dans les portées qu'elles produisent, en raison de l'espacement irrégulier des saisons de reproduction. Ainsi, lorsqu'elles produisent une portée d'été, les femelles ont la possibilité de produire une nouvelle portée beaucoup plus rapidement que lorsqu'elles produisent des portées au printemps. Ainsi, les femelles pourraient diminuer leur investissement reproducteur dans les portées d'été par rapport aux portées de printemps, ce qui aurait des effets sur le phénotype des jeunes. Il serait donc très intéressant d'analyser ces effets dans la population des monts Sutton. Cependant, de tels effets maternels sont susceptibles d'être confondus avec les bases génétiques des traits affectés. Les jeunes issus d'une même portée peuvent avoir un phénotype similaire parce qu'ils se sont développés dans un environnement semblable, ou encore parce qu'ils partagent les mêmes gènes (Wilson et al. 2009). Ainsi une approche expérimentale, manipulant les conditions de développement des jeunes, ou encore une approche de génétique quantitative comparant le phénotype des individus possédant un lien de parenté permettraient de quantifier la contribution des différences sources de variation à la diversité de patrons d'exploration et de reproduction observées chez le tamia rayé (Lynch et Walsh, 1998; Kruuk, 2004; Wil-

son et al., 2009).

BIBLIOGRAPHIE

Angelier, F., B. Moe, H. Weimerskirch, & O. Chastel. 2007a. « Age-specific reproductive success in a long-lived bird : do older parents resist stress better ? », *Journal of Animal Ecology*, vol. 76, no. 6, p. 1181–1191.

Angelier, F., S. A. Shaffer, H. Weimerskirch, & O. Chastel. 2006. « Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird : the wandering albatross. », *General and Comparative Endocrinology*, vol. 149, no. 1, p. 1–9.

Angelier, F., S. A. Shaffer, H. Weimerskirch, C. Trouve, & O. Chastel. 2007b. « Corticosterone and foraging behavior in a pelagic seabird », *Physiological and Biochemical Zoology*, vol. 80, no. 3, p. 283–292.

Archer, J. 1973. « Tests for emotionality in rats and mice : a review », *Animal Behaviour*, vol. 21, no. 2, p. 205–235.

———. 1975. « Rodent sex differences in emotional and related behavior », *Behavioral Biology*, vol. 14, no. 4, p. 451–479.

Astheimer, L. B., W. A. Buttemer, & J. C. Wingfield. 1992. « Interactions of corticosterone with feeding, activity and metabolism in passerine birds », *Ornis Scandinavica*, vol. 23, no. 3, p. 355–365.

———. 1994. « Gender and seasonal differences in the adrenocortical response to ACTH challenge in an arctic passerine, *Zonotrichia leucophrys gambelii* », *General and Comparative Endocrinology*, vol. 43, no. 1, p. 39–47.

Atwell, J. W., G. C. Cardoso, D. J. Whittaker, S. Campbell-Nelson, K. W. Robertson, & E. D. Ketterson. 2012. « Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. », *Behavioral Ecology*, vol. 23, no. 5, p. 960–969.

Barbazanges, A., P. V. Piazza, M. LeMoal, & S. Maccari. 1996. « Maternal glucocorticoid secretion mediates long-term effects of prenatal stress », *Journal of Neuroscience*, vol. 16, no. 12, p. 3943–3949.

Bates, D., M. Maechler, & B. Bolker. 2011. *lme4 : Linear mixed-effects models using S4 classes.*

Baugh, A. T., S. V. Schaper, M. Hau, J. F. Cockrem, P. de Goede, & K. van Oers. 2012. « Corticosterone responses differ between lines of great tits (*Parus major*) selected for divergent personalities », *General and Comparative Endocrinology*, vol. 175, no. 3, p. 488–494.

Bell, A. M. 2005. « Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*) », *Journal of Evolutionary Biology*, vol. 18, no. 2, p. 464–473.

Benus, R. F., B. Bohus, J. M. Koolhaas, & G. A. van Oortmerssen. 1989. « Behavioral strategies of aggressive and non-aggressive mice in active shock avoidance », *Behavioural Processes*, vol. 20, no. 1, p. 1–12.

Benus, R. F., B. Bohus, & G. A. van Oortmerssen. 1991. « Heritable variation for aggression as a reflection of individual coping strategies », *Experientia*, vol. 47, no. 10, p. 1008–1019.

Bergeron, P., P. O. Montiglio, D. Réale, M. M. Humphries, O. Gimenez, & D. Garant. 2013. « Disruptive viability selection on adult exploratory behavior in eastern chipmunks », *Journal of Evolutionary Biology*, vol. 26, no. 4, p. 766–774.

Bergeron, P., D. Réale, M. M. Humphries, & D. Garant. 2011a. « Anticipation and tracking of pulsed resources drive population dynamics in eastern chipmunks », *Ecology*, vol. 92, no. 11, p. 2027–2034.

Bergeron, P., D. Réale, M. M. Humphries, & D. Garant. 2011b. « Evidence of multiple paternity and mate selection for inbreeding avoidance in wild eastern chipmunks », *Journal of Evolutionary Biology*, vol. 24, no. 8, p. 1685–1694.

Bielby, J., G. M. Mace, O. R. P. Bininda-Emonds, M. Cardillo, J. L. Gittleman, K. E. Jones, C. D. L. Orme, & A. Purvis. 2007. « The fast-slow continuum in mammalian life history : an empirical reevaluation », *American Naturalist*, vol. 169, no. 6, p. 748–757.

Biro, P. A. & J. A. Stamps. 2008. « Are animal personality traits linked to life-history productivity ? », *Trends in Ecology and Evolution*, vol. 23, no. 7, p. 361–368.

Blondel, J. 2007. « Coping with habitat heterogeneity : the story of Mediterranean blue tits », *Journal of Ornithology*, vol. 148, no. S1, p. 3–15.

Bókony, V., A. Z. Lendvai, A. Liker, F. Angelier, J. C. Wingfield, & O. Chastel. 2009. « Stress response and the value of reproduction : are birds prudent parents ? », *American Naturalist*, vol. 173, no. 5, p. 589–598.

Bonier, F., I. T. Moore, P. R. Martin, & R. J. Robertson. 2009. « The relationship between fitness and baseline glucocorticoids in a passerine bird. », *General and Comparative Endocrinology*, vol. 163, no. 1-2, p. 208–213.

Boon, A. K., S. Boutin, & D. Réale. 2008. « Personality, habitat use, and their conse-

quences for survival in North American red squirrels *Tamiasciurus hudsonicus* », *Oikos*, vol. 117, no. 9, p. 1321–1328.

Boon, A. K., D. Réale, & S. Boutin. 2007. « The interaction between personality, offspring fitness and food abundance in North American red squirrels », *Ecology Letters*, vol. 10, no. 11, p. 1094–1104.

Boonstra, R. 2005. « Equipped for life : the adaptive role of the stress axis in male mammals », *Journal of Mammalogy*, vol. 86, no. 2, p. 236–247.

Boonstra, R., A. H. Hubbs, E. A. Lacey, & C. J. McColl. 2001. « Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon », *Canadian Journal of Zoology*, vol. 79, no. 1, p. 49–58.

Boonstra, R. & C. J. McColl. 2000. « Contrasting stress response of male arctic ground squirrels and red squirrels », *Journal of Experimental Zoology*, vol. 286, no. 4, p. 390–404.

Bortolotti, G. R., T. A. Marchant, J. Blas, & T. German. 2008. « Corticosterone in feathers is a long-term, integrated measure of avian stress physiology », *Functional Ecology*, vol. 22, no. 3, p. 494–500.

Bosson, C. O., R. Palme, & R. Boonstra. 2009. « Assessment of the stress response in columbian ground squirrels : laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites », *Physiological and Biochemical Zoology*, vol. 82, no. 3, p. 291–301.

Both, C. 1998. « Experimental evidence for density dependence of reproduction in great tits », *Journal of Animal Ecology*, vol. 67, no. 4, p. 667–674.

Both, C. & M. E. Visser. 2000. « Breeding territory size affects fitness : an experimental study on competition at the individual level », *Journal of Animal Ecology*, p. 1021–1030.

Boyer, N., D. Réale, J. Marmet, B. Pisanu, & J.-L. Chapuis. 2010. « Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. », *Journal of Animal Ecology*, vol. 79, no. 3, p. 538–547.

Breuner, C. 2008. « Maternal stress, glucocorticoids, and the maternal/fetal match hypothesis », *Hormones and Behavior*, vol. 54, no. 4, p. 485–487.

Breuner, C. W., S. H. Patterson, & T. P. Hahn. 2008. « In search of relationships between the acute adrenocortical response and fitness », *General and Comparative Endocrinology*, vol. 157, no. 3, p. 288–295.

Breuner, C. W., J. C. Wingfield, & L. M. Romero. 1999. « Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow », *Journal of Experimental Zoology*, vol. 284, no. 3, p. 334–342.

Brockmann, H. J. 2001. « Evolution of alternative strategies and tactics », *Advances in the Study of Behavior*, vol. 30, p. 1–52.

Brockmann, H. J. & M. Taborsky. 2008. *Alternative reproductive tactics and the evolution of alternative allocation phenotypes.* In *Alternative Reproductive Tactics*, Oliviera, R. F., M. Taborsky, & H. J. Brockman, éd. Cambridge : Cambridge University Press.

Burnham, K. P. & D. Anderson. 2002. *Model selection and multi-model inference.* Springer.

Calenge, C. 2006. « The package adehabitat for the R software : a tool for the analysis of space and habitat use by animals », *Ecological Modelling*, vol. 197, no. 3-4, p. 516–519.

Carere, C., P. J. Drent, L. Privitera, J. M. Koolhaas, & T. G. G. Groothuis. 2005. « Personalities in great tits, *Parus major* : stability and consistency », *Animal Behaviour*, vol. 70, no. 4, p. 795–805.

Carere, C., T. G. G. Groothuis, E. Möstl, S. Daan, & J. M. Koolhaas. 2003. « Fecal corticosteroids in a territorial bird selected for different personalities : daily rhythm and the response to social stress », *Hormones and Behavior*, vol. 43, no. 5, p. 540–548.

Carere, C. & K. van Oers. 2004. « Shy and bold great tits (*Parus major*) : body temperature and breath rate in response to handling stress », *Physiology and Behavior*, vol. 82, p. 905–912.

Carlstead, K. & J. L. Brown. 2005. « Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros », *Zoo Biology*, vol. 24, p. 215–232.

Catalani, A., P. Casolini, S. Scaccianoce, F. R. Patacchioli, P. Spinozzi, & L. Angelucci. 2000. « Maternal corticosterone during lactation permanently affects brain corticosteroid receptors, stress response and behaviour in rat progeny », *Neuroscience*, vol. 100, no. 2, p. 319–325.

Cavigelli, S. A., S. L. Monfort, T. K. Whitney, Y. S. Mechref, M. Novotny, & M. K. McClintock. 2005. « Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms », *Journal of Endocrinology*, vol. 184, no. 1, p. 153–163.

Chambers, J. & D. Garant. 2010. « Determinants of population genetic structure in eastern chipmunks (*Tamias striatus*) : the role of landscape barriers and sex-biased dispersal », *Journal of Heredity*, vol. 101, p. 413–422.

Clark, C. W. 1994. « Antipredator behavior and the asset-protection principle », *Behavioral Ecology*, vol. 5, no. 2, p. 159–170.

Cockrem, J. 2007. « Stress, corticosterone responses and avian personalities », *Journal of Ornithology*, vol. 148, no. S2, p. 169–178.

Cook, K. V., C. M. O'Connor, K. M. Gilmour, & S. J. Cooke. 2011. « The glucocorticoid stress response is repeatable between years in a wild teleost fish. », *Journal of Comparative Physiology. Part A, Neuroethology, Sensory, Neural, & Behavioral Physiology*, vol. 197, no. 12, p. 1189–1196.

Cook, K. V., C. M. O'Connor, S. H. McConnachie, K. M. Gilmour, & S. J. Cooke. 2012. « Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. », *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, vol. 161, no. 3, p. 337–343.

Coppens, C. M., S. F. de Boer, & J. M. Koolhaas. 2010. « Coping styles and behavioural flexibility : towards underlying mechanisms. », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 365, no. 1560, p. 4021–4028.

Corbett, B., C. W. Schupp, S. Levine, & S. Mendoza. 2009. « Comparing cortisol, stress, and sensory sensitivity in children with autism. », *Autism Research*, vol. 2, no. 1, p. 39–49.

Crawley, M. J. 2007. *The R Book*. Wiley.

Dantzer, B., A. G. McAdam, R. Palme, Q. E. Fletcher, S. Boutin, M. M. Humphries, & R. Boonstra. 2010. « Fecal cortisol metabolite levels in free-ranging North American red squirrels : assay validation and the effects of reproductive condition », *General and Comparative Endocrinology*, vol. 167, no. 2, p. 279–286.

Darwin, C. 1859. *On the origin of species*. « The work of Charles Darwin », no 15. New York University Press.

Dingemanse, N. J., C. Both, P. J. Drent, K. van Oers, & A. J. van Noordwijk. 2002. « Repeatability and heritability of exploratory behaviour in great tits from the wild », *Animal Behaviour*, vol. 64, no. 6, p. 929–938.

Dingemanse, N. J., K. M. Bouwman, M. van de Pol, T. van Overveld, S. C. Patrick, E. Matthysen, & J. L. Quinn. 2012. « Variation in personality and behavioural plasticity across four populations of the great tit *Parus major*. », *Journal of Animal Ecology*, vol. 81, no. 1, p. 116–126.

Dingemanse, N. J. & P. de Goede. 2004. « The relation between dominance and exploratory behavior is context-dependent in wild great tits », *Behavioral Ecology*, vol. 15, no. 6, p. 1023–1030.

Dingemanse, N. J. & M. Wolf. 2010. « Recent models for adaptive personality differences : a review », *Philosophical Transactions of the Royal Society. Series B,*

Biological Sciences, vol. 365, no. 1560, p. 3947–3958.

Dingemanse, N. J., J. Wright, A. J. N. Kazem, D. K. Thomas, R. Hickling, & N. Dawnay. 2007. « Behavioural syndromes differ predictably between 12 populations of three-spined stickleback », *Journal of Animal Ecology*, vol. 76, no. 6, p. 1128–1138.

Dubuc Messier, G.and Garant, D., P. Bergeron, & D. Réale. 2012. « Environmental conditions affect spatial genetic structures and dispersal patterns in a solitary rodent », *Molecular Ecology*, vol. 21, p. 5363–5373.

Dufty, A. M., J. Clobert, P. Anders, A. M. Dufty, & A. P. Moller. 2002. « Hormones, developmental plasticity and adaptation », *Trends in Ecology and Evolution*, vol. 17, no. 4, p. 190–196.

Dunford, C. 1970. « Behavioral aspects of spatial organization in the chipmunk, Tamias striatus », *Behaviour*, vol. 36, no. 3, p. 215–231.

Elliott, L. 1978. « Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack Mountains », *Smithsonian Contributions to Zoology*, vol. 265.

Felszeghy, K., G. Bagday, & C. Nyakas. 2000. « Blunted pituitary-adrenocortical stress response in adult rats following neonatal dexamethasone treatment », *Journal of Neuroendocrinology*, vol. 12, no. 10, p. 1014–1021.

Fletcher, Q. E., J. R. Speakman, S. Boutin, A. G. McAdam, S. B. Woods, & M. M. Humphries. 2012. « Seasonal stage differences overwhelm environmental and individual factors as determinants of energy expenditure in free-ranging red squirrels », *Functional Ecology*, vol. 26, no. 3, p. 677–687.

Fox, C. W., D. A. Roff, & D. J. Fairbairn. 2001. *Evolutionary Ecology*. New York : Oxford University Press.

French, A. R. 2000. « Interdependency of stored food and changes in body temperature during hibernation of the eastern chipmunk, *Tamias striatus* », *Journal of Mammalogy*, vol. 81, no. 4, p. 979–985.

Frigerio, D., J. Dittami, E. Möstl, & K. Kotrschal. 2004. « Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (Anser anser). », *General and Comparative Endocrinology*, vol. 137, no. 1, p. 29–36.

Fucikova, E., P. J. Drent, N. Smits, & K. van Oers. 2009. « Handling stress as a measurement of personality in great tit nestlings (*Parus major*) », *Ethology*, vol. 115, no. 4, p. 366–374.

Gaillard, J. M., D. Pontier, D. Allainé, J. D. Lebreton, J. Trouvilliez, & J. Clobert. 1989. « An analysis of demographic tactics in birds and mammals », *Oikos*, vol. 56, no. 1, p. 59–76.

Galea, L. A., M. Kavaliers, & K. P. Ossenkopp. 1996. « Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus* », *Journal of Experimental Biology*, vol. 199, no. 1, p. 195–200.

Goymann, W. 2005. « Noninvasive monitoring of hormones in bird droppings - physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels », *Annals of the New York Academy of Sciences*, vol. 1046, no. 1, p. 35–53.

Gutman, R., T. Dayan, O. Levy, I. Schubert, & N. Kronfeld-Schor. 2011. « The effect of the lunar cycle on fecal cortisol metabolite levels and foraging ecology of nocturnally and diurnally active spiny mice », *Plos One*, vol. 6, no. 8, p. e23446.

Hall, C. L., M. M. Humphries, & D. L. Kramer. 2007. « Resource tracking by eastern chipmunks : the sampling of renewing patches », *Canadian Journal of Zoology*, vol. 85, no. 4, p. 536–548.

Harper, J. M. & S. N. Austad. 2000. « Fecal glucocorticoids : a noninvasive method of measuring adrenal activity in wild and captive rodents », *Physiological and Biochemical Zoology*, vol. 73, no. 1, p. 12–22.

Harris, S., I. W. Ramnarine, H. G. Smith, & L. B. Pettersson. 2010. « Picking personalities apart : estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata* », *Oikos*, vol. 119, no. 11, p. 1711–1718.

Heidinger, B. J., I. C. T. Nisbet, & E. D. Ketterson. 2006. « Older parents are less responsive to a stressor in a long-lived seabird : a mechanism for increased reproductive performance with age ? », *Proceedings of the Royal Society. Series B, Biological Sciences*, vol. 273, no. 1598, p. 2227–2231.

———. 2008. « Changes in adrenal capacity contribute to a decline in the stress response with age in a long-lived seabird. », *General and Comparative Endocrinology*, vol. 156, no. 3, p. 564–568.

Heistermann, M., R. Palme, & A. Ganswindt. 2006. « Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis », *American Journal of Primatology*, vol. 68, no. 3, p. 257–273.

Holberton, R. L. & J. C. Wingfield. 2003. « Modulating the corticosterone stress response : a mechanism for balancing individual risk and reproductive success in arctic-breeding sparrows ? », *Auk*, vol. 120, no. 4, p. 1140–1150.

Houston, A. I. 2010. « Evolutionary models of metabolism, behaviour and personality. », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 365, no. 1560, p. 3969–3975.

Humphries, M. M., D. W. Thomas, & D. L. Kramer. 2001. « Torpor and digestion in

food-storing hibernators », *Physiological and Biochemical Zoology*, vol. 74, no. 2, p. 283–292.

Hutt, C. 1969. « Exploration, arousal and autism », *Psychological Research*, vol. 33, no. 1, p. 1–8.

Jarvis, S., C. Moinard, S. K. Robson, E. Baxter, E. Ormandy, A. J. Douglas, J. R. Seckl, J. A. Russell, & A. B. Lawrence. 2006. « Programming the offspring of the pig by prenatal social stress : neuroendocrine activity and behaviour », *Hormones and Behavior*, vol. 49, no. 1, p. 68–80.

Jessop, T. S. 2001. « Modulation of the adrenocortical stress response in marine turtles (Cheloniidae) : evidence for a hormonal tactic maximizing maternal reproductive investment », *Journal of Zoology*, vol. 254, no. 1, p. 57–65.

Jozet-Alves, C., J. Modéran, & L. Dickel. 2008. « Sex differences in spatial cognition in an invertebrate : the cuttlefish. », *Proceedings of the Royal Society. Series B, Biological Sciences*, vol. 275, no. 1646, p. 2049–2054.

Jurke, M. H., N. M. Czekala, D. G. Lindburg, & S. E. Millard. 1997. « Fecal corticoid metabolite measurement in the cheetah (*Acinonyx jubatus*) », *Zoo Biology*, vol. 16, no. 2, p. 133–147.

Kalinowski, S. T., M. L. Taper, & T. C. Marshall. 2007. « Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. », *Molecular Ecology*, vol. 16, no. 5, p. 1099–1106.

Kenagy, G. J. & N. J. Place. 2000. « Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks : effects of reproduction and capture and handling », *General and Comparative Endocrinology*, vol. 117, no. 2, p. 189–199.

Kitaysky, A. S., E. V. Kitaiskaia, J. C. Wingfield, & J. F. Piatt. 2001. « Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks », *Journal of Comparative Physiology. Part B, Biochemical Systemic & Environmental Physiology*, vol. 171, no. 8, p. 701–709.

Kitaysky, A. S., J. F. Piatt, S. A. Hatch, E. V. Kitaiskaia, Z. M. Benowitz-Fredericks, M. T. Shultz, & J. C. Wingfield. 2010. « Food availability and population processes : severity of nutritional stress during reproduction predicts survival of long-lived seabirds », *Functional Ecology*, vol. 24, no. 3, p. 625–637.

Kitaysky, A. S., J. F. Piatt, J. C. Wingfield, & M. Romano. 1999. « The adrenocortical stress-response of Black-legged Kittiwake chicks in relation to dietary restrictions », *Journal of Comparative Physiology. Part B, Biochemical Systemic & Environmental Physiology*, vol. 169, no. 4-5, p. 303–310.

Kitaysky, A. S., J. C. Wingfield, & J. F. Piatt. 1999. « Dynamics of food availability, body condition and physiological stress response in breeding Black-legged

Kittiwakes », *Functional Ecology*, vol. 13, no. 5, p. 577–584.

Koford, R. R. 1982. « Mating system of a territorial tree squirrel (*Tamiasciurus douglasii*) in California », *Journal of Mammalogy*, vol. 63, no. 2, p. 274–283.

Koolhaas, J. M., S. F. de Boer, C. M. Coppens, & B. Buwalda. 2010. « Neuroendocrinology of coping styles : towards understanding the biology of individual variation », *Frontiers in Neuroendocrinology*, vol. 31, no. 3, p. 307–321.

Koolhaas, J. M., S. M. Korte, S. F. De Boer, B. J. Van Der Vegt, C. G. Van Reenen, H. Hopster, I. C. De Jong, M. A. W. Ruis, & H. J. Blokhuis. 1999. « Coping styles in animals : current status in behavior and stress-physiology », *Neuroscience and Biobehavioral Reviews*, vol. 23, no. 7, p. 925–935.

Koprowski, J. L. 1993. « Alternative reproductive tactics in male eastern gray squirrels : "making the best of a bad job" », *Behavioral Ecology*, vol. 4, no. 2, p. 165–171.

Krause, E. T. & T. Liesenjohann. 2012. « Predation pressure and food abundance during early life alter risk-taking behaviour and growth of guppies (*Poecilia reticulata*) », *Behaviour*, vol. 149, no. 1, p. 1–14.

Kruuk, L. E. B. 2004. « Estimating genetic parameters in natural populations using the 'animal model' », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 359, no. 1446, p. 873–890.

Lancaster, L. T., L. C. Hazard, J. Clobert, & B. R. Sinervo. 2008. « Corticosterone manipulation reveals differences in hierarchical organization of multidimensional reproductive trade-offs in r-strategist and K-strategist females », *Journal of Evolutionary Biology*, vol. 21, no. 2, p. 556–565.

Landry-Cuerrier, M., D. Munro, D. W. Thomas, & M. M. Humphries. 2008. « Climate and resource determinants of fundamental and realized metabolic niches of hibernating chipmunks. », *Ecology*, vol. 89, no. 12, p. 3306–3316.

Legendre, P. & L. Legendre. 2011. *Numerical Ecology.* « Developments in environmental modelling », no 20. Elsevier.

Lendvai, A. Z. & O. Chastel. 2008. « Experimental mate-removal increases the stress response of female house sparrows : the effects of offspring value ? », *Hormones and Behavior*, vol. 53, p. 395–401.

Lepschy, M., C. Touma, R. Hruby, & R. Palme. 2007. « Non-invasive measurement of adrenocortical activity in male and female rats », *Laboratory Animals*, vol. 41, no. 1016, p. 372–387.

Lessels, C. M. & P. T. Boag. 1987. « Unrepeatable repeatabilities : a common mistake », *Auk*, vol. 104, no. 1, p. 116–121.

Loew, S. S. 1999. « Sex-biased dispersal in eastern chipmunks, *Tamias striatus* », *Evo-*

lutionary Ecology, no. 1, p. 557–577.

Lordi, B., V. Patin, P. Protais, D. Mellier, & J. Caston. 2000. « Chronic stress in pregnant rats : effects on growth rate, anxiety and memory capabilities of the offspring », *International Journal of Psychophysiology*, vol. 37, no. 2, p. 195–205.

Love, O. P., E. H. Chin, K. E. Wynne-Edwards, & T. D. Williams. 2005. « Stress hormones : a link between maternal condition and sex-biaised reproductive investment », *American Naturalist*, vol. 166, p. 751–766.

Love, O. P. & T. D. Williams. 2008a. « Plasticity in the adrenocortical response of a free-living vertebrate : the role of pre- and post-natal developmental stress », *Hormones and Behavior*, vol. 54, no. 4, p. 496–505.

———. 2008b. « The adaptive value of stress-induced phenotypes : effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness », *American Naturalist*, vol. 172, no. 4, p. E135–E149.

Lynch, J. W., T. E. Ziegler, & K. B. Strier. 2002. « Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys, *Cebus apella nigritus* », *Hormones and Behavior*, vol. 41, no. 3, p. 275–287.

Lynch, M. & B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates Incorporated.

Macbeth, B. J., M. R. L. Cattet, G. B. Stenhouse, M. L. Gibeau, & D. M. Janz. 2010. « Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*) : considerations with implications for other wildlife », *Canadian Journal of Zoology*, vol. 88, no. 10, p. 935–949.

Maccari, S. 1995. « Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress », *Journal of Neuroscience*, vol. 15, no. 1, p. 110–116.

Mangel, M. & J. Stamps. 2001. « Trade-offs between growth and mortality and the maintenance of individual variation in growth », *Evolutionary Ecology Research*, vol. 3, no. 5, p. 583–593.

Martin, J. G. A. & D. Réale. 2008. « Animal temperament and human disturbance : implications for the response of wildlife to tourism », *Behavioural Processes*, vol. 77, no. 1, p. 66–72.

Mateo, J. M. 2007. « Ecological and hormonal correlates of antipredator behavior in adult Belding's ground squirrels (*Spermophilus beldingi*) », *Behavioral Ecology and Sociobiology*, vol. 62, no. 1, p. 37–49.

McGlothlin, J. W. & E. D. Ketterson. 2008. « Hormone-mediated suites as adaptations and evolutionary constraints », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 363, no. 1497, p. 1611–1620.

Montiglio, P.-O., D. Garant, F. Pelletier, & D. Réale. 2012a. « Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunks, *Tamias striatus* », *Animal Behaviour*, vol. 84, no. 4, p. 1071–1079.

Montiglio, P.-O., D. Garant, D. W. Thomas, & D. Réale. 2010. « Individual variation in temporal activity patterns in open-field tests », *Animal Behaviour*, vol. 80, p. 905–912.

Montiglio, P.-O., F. Pelletier, R. Palme, D. Garant, D. Réale, & R. Boonstra. 2012b. « Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk (*Tamias striatus*) : validation and comparison of two enzyme immunoassays. », *Physiological and Biochemical Zoology*, vol. 85, no. 2, p. 183–193.

Morrow, C. J., E. S. Kolver, G. A. Verkerk, & L. R. Matthews. 2002. « Fecal glucocorticoid metabolites as a measure of adrenal activity in dairy cattle », *General and Comparative Endocrinology*, vol. 126, no. 2, p. 229–241.

Möstl, E. & R. Palme. 2002. « Hormones as indicators of stress », *Domestic Animal Endocrinology*, vol. 23, no. 1-2, p. 67–74.

Möstl, E., S. Rettenbacher, & R. Palme. 2005. « Measurement of corticosterone metabolites in birds' droppings : an analytical approach », *Annals of the New York Academy of Sciences*, vol. 1046, no. 1, p. 17–34.

Munro, D., D. W. Thomas, & M. M. Humphries. 2008. « Extreme suppression of aboveground activity by a food-storing hibernator, the eastern chipmunk (*Tamias striatus*) », *Canadian Journal of Zoology*, vol. 86, no. 5, p. 364–370.

Naya, D. E., L. A. Ebensperger, P. Sabat, & F. Bozinovic. 2008. « Digestive and metabolic flexibility allows female degus to cope with lactation costs », *Physiological and Biochemical Biology*, vol. 81, p. 186–194.

Nicolaus, M., J. M. Tinbergen, K. M. Bouwman, S. P. M. Michler, R. Ubels, C. Both, B. Kempenaers, & N. J. Dingemanse. 2012. « Experimental evidence for adaptive personalities in a wild passerine bird. », *Proceedings of the Royal Society. Series B, Biological Sciences*, vol. In press.

Nussey, D. H., L. E. B. Kruuk, A. Donald, M. Fowlie, & T. H. Clutton-Brock. 2006. « The rate of senescence in maternal performance increases with early-life fecundity in red deer », *Ecology Letters*, vol. 9, no. 12, p. 1342–1350.

O'Reilly, K. M. & J. C. Wingfield. 2001. « Ecological factors underlying the adrenocortical response to capture stress in arctic-breeding shorebirds. », *General and Comparative Endocrinology*, vol. 124, no. 1, p. 1–11.

Otte, C., S. Hart, T. C. Neylan, C. R. Marmar, K. Yaffe, & D. C. Mohr. 2005. « A meta-analysis of cortisol response to challenge in human aging : importance of

gender. », *Psychoneuroendocrinology*, vol. 30, no. 1, p. 80–91.

Overli, O., S. Kotzian, & S. Winberg. 2002. « Effects of cortisol on aggression and locomotor activity in rainbow trout », *Hormones and Behavior*, vol. 42, no. 1, p. 53–61.

Overli, O., C. Sorensen, K. G. T. Pulman, T. G. Pottinger, W. Korzan, C. H. Summers, & G. E. Nilsson. 2007. « Evolutionary background for stress-coping styles : relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates », *Neuroscience and Biobehavioral Reviews*, vol. 31, no. 3, p. 396–412.

Overli, O., S. Winberg, & T. G. Pottinger. 2005. « Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout–a review. », *Integrative and Comparative Biology*, vol. 45, no. 3, p. 463–474.

Palme, R. 2005. « Measuring fecal steroids - guidelines for practical application », *Annals of the New York Academy of Sciences*, vol. 1046, no. 1, p. 75–80.

Palme, R. & E. Möstl. 1997. « Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood », *Zeitschrift Fur Saugetierkunde - International Journal of Mammalian Biology*, vol. 62, p. S192–S197.

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr, & E. Möstl. 2005. « Stress hormones in mammals and birds - comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples », *Trends in Comparative Endocrinology and Neurobiology*, vol. 1040, no. 1, p. 162–171.

Patterson, L. D. & A. I. Schulte-Hostedde. 2011. « Behavioural correlates of parasitism and reproductive success in male eastern chipmunks, *Tamias striatus* », *Animal Behaviour*, vol. 81, no. 6, p. 1129–1137.

Perrin, N. & J. Travis. 1992. « On the use of constraints in evolutionary biology and some allergic reactions to them », *Functional Ecology*, vol. 6, no. 3, p. 361–363.

Pidduck, E. R. & J. B. Falls. 1973. « Reproduction and emergence of juveniles in *Tamias striatus* (Rodentia : Sciuridae) at two localities in Ontario, Canada », *Journal of Mammalogy*, vol. 54, no. 3, p. 693–707.

Pinheiro, J. C. & D. M. Bates. 2000. *Mixed effects models in S and S-Plus*. New York : Springer-Verlag.

Place, N. J. & G. J. Kenagy. 2000. « Seasonal changes in plasma testosterone and glucocorticosteroids in free-living male yellow-pine chipmunks and the response to capture and handling », *Journal of Comparative Physiology. Part B, Biochemical, Systemic & Environmental Physiology*, vol. 170, no. 3, p. 245–251.

Pravosudov, V. V. 2003. « Long-term moderate elevation of corticosterone facilitates avian food-caching behaviour and enhances spatial memory », *Proceedings of the*

Royal Society. Series B, Biological Sciences, vol. 274, p. 2599–2605.

Pravosudov, V. V., A. S. Kitaysky, J. C. Wingfield, & N. S. Clayton. 2001. « Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). », *General and Comparative Endocrinology*, vol. 123, no. 3, p. 324–331.

Promislow, D. E. L. & P. H. Harvey. 1990. « Living fast and dying young : a comparative analysis of life-history variation among mammals », *Journal of Zoology*, vol. 220, no. 3, p. 417–437.

Quinn, J. L., S. C. Patrick, S. Bouwhuis, T. A. Wilkin, & B. C. Sheldon. 2009. « Heterogeneous selection on a heritable temperament trait in a variable environment », *Journal of Animal Ecology*, vol. 78, no. 6, p. 1203–1215.

R Development Core Team. 2011. « R : a language and environment for statistical computing ».

Réale, D., N. J. Dingemanse, A. J. N. Kazem, & J. Wright. 2010a. « Evolutionary and ecological approaches to the study of personality. », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 365, no. 1560, p. 3937–3946.

Réale, D. & M. Festa-Bianchet. 2003. « Predator-induced natural selection on temperament in bighorn ewes », *Animal Behaviour*, vol. 65, no. 3, p. 463–470.

Réale, D., B. Y. Gallant, M. Leblanc, & M. Festa-Bianchet. 2000. « Consistency of temperament in bighorn ewes and correlates with behaviour and life history », *Animal Behaviour*, vol. 60, no. 5, p. 589–597.

Réale, D., D. Garant, M. M. Humphries, P. Bergeron, V. Careau, & P.-O. Montiglio. 2010b. « Personality and the emergence of the pace-of-life syndrome concept at the population level. », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 365, no. 1560, p. 4051–4063.

Réale, D., S. M. Reader, D. Sol, P. T. McDougall, & N. J. Dingemanse. 2007. « Integrating animal temperament within ecology and evolution », *Biological Reviews*, vol. 82, no. 2, p. 291–318.

Reeder, D. M. & K. M. Kramer. 2005. « Stress in free-ranging mammals : integrating physiology, ecology, and natural history », *Journal of Mammalogy*, vol. 86, no. 2, p. 225–235.

Ricklefs, R. E. & M. Wikelski. 2002. « The physiology/life-history nexus », *Trends in Ecology and Evolution*, vol. 17, no. 10, p. 462–468.

Rimondini, R., G. Agren, S. Borjesson, W. Sommer, & M. Heilig. 2003. « Persistent behavioral and autonomic supersensitivity to stress following prenatal stress exposure in rats », *Behavioural Brain Research*, vol. 140, no. 1-2, p. 75–80.

Roff, D. & D. J. Fairbairn. 2007. « The evolution of trade-offs : where are we ? », *Journal of Evolutionary Biology*, vol. 20, no. 2, p. 433–447.

Romero, L. M. 2002. « Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates », *General and Comparative Endocrinology*, vol. 128, no. 1, p. 1–24.

———. 2004. « Physiological stress in ecology : lessons from biomedical research », *Trends in Ecology and Evolution*, vol. 19, no. 5, p. 249–255.

Romero, L. M., M. J. Dickens, & N. E. Cyr. 2009. « The reactive scope model - a new model integrating homeostasis, allostasis, and stress », *Hormones and Behavior*, vol. 55, no. 3, p. 375–389.

Romero, L. M., M. Ramenofsky, & J. C. Wingfield. 1997. « Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*) », *Comparative Biochemistry and Physiology. Part C, Pharmacology, Toxicology & Endocrinology*, vol. 116, p. 171–177.

Romero, L. M. & M. Wikelski. 2001. « Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events », *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, p. 7366–7370.

———. 2010. « Stress physiology as a predictor of survival in Galapagos marine iguanas. », *Proceedings of the Royal Society of London. Series B, Biological Sciences*, vol. 277, no. 1697, p. 3157–3162.

Sapolsky, R. M. & J. Ray. 1989. « Style of dominance and their endocrine correlates among wild baboons (*Papio anubis*) », *American Journal of Primatology*, vol. 18, p. 1–12.

Sapolsky, R. M., L. M. Romero, & A. U. Munck. 2000. « How do glucocorticoids influence stress responses ? integrating permissive, suppressive, stimulatory, and preparative actions », *Endocrine Reviews*, vol. 21, no. 1, p. 55–89.

Schjolden, J., T. Backström, K. G. T. Pulman, T. G. Pottinger, & S. Winberg. 2005. « Divergence in behavioural responses to stress in two strains of rainbow trout (*Oncorhynchus mykiss*) with contrasting stress responsiveness. », *Hormones and Behavior*, vol. 48, no. 5, p. 537–544.

Schulte-Hostedde, A. I. & J. S. Millar. 2002. « "Little chipmunk" syndrome ? male body size and dominance in captive yellow-pine chipmunks (*Tamias amoenus*) », *Ethology*, vol. 108, no. 2, p. 127–137.

Schulte-Hostedde, A. I., J. S. Millar, & H. L. Gibbs. 2002. « Female-biased sexual size dimorphism in the yellow-pine chipmunk (*Tamias amoenus*) : sex-specific

patterns of annual reproductive success and survival », *Evolution*, vol. 56, no. 12, p. 2519–2529.

Seckl, J. R. 2001. « Glucocorticoid programming of the fetus : adult phenotypes and molecular mechanisms », *Molecular and Cellular Endocrinology*, vol. 185, no. 1-2, p. 61–71.

Sheriff, M., B. Dantzer, B. Delhanty, R. Palme, & R. Boonstra. 2011a. « Measuring stress in wildlife : techniques for quantifying glucocorticoids », *Oecologia*, vol. 166, p. 869–887.

Sheriff, M. J., S. A. Donker, H. Wheeler, C. J. Krebs, R. Palme, D. S. Hik, & R. Boonstra. 2011b. « Mountain top and valley bottom experiences : the stress axis as an integrator of environmental variability in arctic ground squirrel populations », *Journal of Zoology, submitted.*

Sheriff, M. J., C. J. Krebs, & R. Boonstra. 2010. « The ghosts of predators past : population cycles and the role of maternal programming under fluctuating predation risk », *Ecology*, vol. 91, no. 10, p. 2983–2994.

———. 2011. « From process to pattern : how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle », *Oecologia*, vol. 166, p. 593–605.

Sih, A. & A. M. Bell. 2008. « Insights for behavioral ecology from behavioral syndromes », *Advances in the Study of Behavior*, vol. 38, no. 8, p. 227–281.

Sih, A., A. M. Bell, & J. C. Johnson. 2004. « Reply to Neff and Sherman. Behavioral syndromes versus darwinian algorithms », *Trends in Ecology and Evolution*, vol. 19, no. 12, p. 622–623.

Sih, A., L. B. Kats, & E. F. Maurer. 2003. « Behavioral correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system », *Animal Behaviour*, vol. 65, no. 1, p. 29–44.

Smith, B. R. & D. T. Blumstein. 2008. « Fitness consequences of personality : a meta-analysis », *Behavioral Ecology*, vol. 19, no. 2, p. 448–455.

Smith, J. E., R. Monclús, D. Wantuck, G. L. Florant, & D. T. Blumstein. 2012. « Fecal glucocorticoid metabolites in wild yellow-bellied marmots : experimental validation, individual differences and ecological correlates. », *General and Comparative Endocrinology*, vol. 178, no. 2, p. 417–426.

Snyder, D. P. 1982. « *Tamias striatus* », *Mammalian Species*, vol. 168, p. 1–8.

Stamps, J. A. 2007. « Growth-mortality tradeoffs and "personality traits" in animals », *Ecology Letters*, vol. 10, no. 5, p. 355–363.

Stearns, S. C. 1992. *The Evolution of Life Histories.* New York : Oxford University

Press.

Strauss, A., E. Mascher, R. Palme, & E. Millesi. 2007. « Sexually mature and immature yearling male European ground squirrels : a comparison of behavioral and physiological parameters », *Hormones and Behavior*, vol. 52, p. 646–652.

Thiel, D., S. Jenni-Eiermann, V. Braunisch, R. Palme, & L. Jenni. 2008. « Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus* : a new methodological approach », *Journal of Applied Ecology*, vol. 45, no. 3, p. 845–853.

Thiel, D., S. Jenni-Eiermann, R. Palme, & L. Jenni. 2011. « Winter tourism increases stress hormone levels in endangered capercaillie *Tetrao urogallus* », *Ibis*, vol. 153, p. 122–133.

Torres Dowdall, J., C. A. Handelsman, E. W. Ruell, S. K. Auer, D. N. Reznick, & C. K. Ghalambor. 2012. « Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies », *Functional Ecology*, vol. 26, p. 616–627.

Touma, C. & R. Palme. 2005. « Measuring fecal glucocorticoid metabolites in mammals and birds : the importance of validation », *Annals of the New York Academy of Sciences*, vol. 1046, no. 1, p. 54–74.

Touma, C., R. Palme, & N. Sachser. 2004. « Analyzing corticosterone metabolites in fecal samples of mice : a noninvasive technique to monitor stress hormones », *Hormones and Behavior*, vol. 45, p. 10–22.

Touma, C., N. Sachser, E. Möstl, & R. Palme. 2003. « Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice », *General and Comparative Endocrinology*, vol. 130, no. 3, p. 267–278.

Travers, M., M. Clinchy, L. Zanette, R. Boonstra, & T. D. Williams. 2010. « Indirect predator effects on clutch size and the cost of egg production », *Ecology Letters*, vol. 13, no. 8, p. 980–988.

van de Pol, M. V. & S. Verhulst. 2006. « Age-dependent traits : a new statistical model to separate within- and between-individual effects », *American Naturalist*, vol. 167, p. 766–773.

Visser, E. K., C. G. van Reenen, J. T. N. van der Werf, M. B. H. Schilder, J. H. Knaap, A. Barneveld, & H. J. Blokhuis. 2002. « Heart rate and heart rate variability during a novel object test and a handling test in young horses », *Physiology and Behavior*, vol. 76, no. 2, p. PII S0031–9384(02)00698–4.

von Merten, S. & B. M. Siemers. 2012. « Exploratory behaviour in shrews : fast-lived *Sorex* versus slow-lived *Crocidura* », *Animal Behaviour*, vol. 84, no. 1, p. 29–38.

Walsh, M. R. & D. N. Reznick. 2009. « Phenotypic diversification across an environ-

mental gradient : a role for predators and resource availability on the evolution of life histories », *Evolution*, vol. 63, p. 3201–3213.

Wasser, S. K., K. E. Hunt, J. L. Brown, K. Cooper, C. M. Crockett, U. Bechert, J. J. Millspaugh, S. Larson, & S. L. Monfort. 2000. « A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species », *General and Comparative Endocrinology*, vol. 120, no. 3, p. 260–275.

Weladji, R. B., J.-M. Gaillard, N. G. Yoccoz, O. Holand, A. Mysterud, A. Loison, M. Nieminen, & N. C. Stenseth. 2006. « Good reindeer mothers live longer and become better in raising offspring. », *Proceedings of the Royal Society. Series B, Biological Sciences*, vol. 273, no. 1591, p. 1239–1244.

Wiersma, P., A. Munoz-Garcia, A an Walker, & J. B. Williams. 2007. « Tropical birds have a slow pace of life », *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, p. 9340–9345.

Wiersma, P., B. Nowak, & J. B. Williams. 2012. « Small organ size contributes to the slow pace of life in tropical birds », *Journal of Experimental Biology*, vol. 215, no. 10, p. 1662–1669.

Williams, G. C. 1966. « Natural Selection, the costs of reproduction, and a refinement of Lack' s principle », *American Naturalist*, vol. 100, no. 916, p. 687–690.

Williams, T. D. 2008. « Individual variation in endocrine systems : moving beyond the 'tyranny of the Golden Mean' », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 363, no. 1497, p. 1687–1698.

Wilson, A. J., D. Réale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. B. Kruuk, & D. H. Nussey. 2009. « An ecologist's guide to the animal model », *Journal of Animal Ecology*, vol. 79, no. 1, p. 13–26.

Wilson, D. S. 1998. « Adaptive individual differences within single populations », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 353, no. 1366, p. 199–205.

Wingfield, J. C. 2005. « The concept of allostasis : coping with a capricious environment », *Journal of Mammalogy*, vol. 86, no. 2, p. 248–254.

Wingfield, J. C., P. Deviche, S. Sharbaugh, L. B. Astheimer, R. Holberton, R. Suydam, & K. Hunt. 1994. « Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska », *Journal of Experimental Zoology*, vol. 270, no. 4, p. 372–380.

Wingfield, J. C. & A. S. Kitaysky. 2002. « Endocrine responses to unpredictable environmental events : stress or anti-stress hormones ? », *Integrative and Comparative Biology*, vol. 42, no. 3, p. 600–609.

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, &

R. D. Richardson. 1998. « Ecological bases of hormone-behavior interactions : the "emergency life history stage" », *American Zoologist*, vol. 38, no. 1, p. 191–206.

Wingfield, J. C. & R. M. Sapolsky. 2003. « Reproduction and resistance to stress : when and how », *Journal of Endocrinology*, vol. 15, no. 8, p. 711–724.

Wingfield, J. C., M. E. Visser, & T. D. Williams. 2008. « Integration of ecology and endocrinology in avian reproduction : a new synthesis », *Philosophical Transactions of the Royal Society. Series B, Biological Sciences*, vol. 363, no. 1497, p. 1581–1588.

Wolf, M., G. S. van Doorn, O. Leimar, & F. J. Weissing. 2007. « Life-history trade-off favour the evolution of animal personalities », *Nature*, vol. 447, no. 7144, p. 581–584.

Wolf, M., G. S. van Doorn, & F. J. Weissing. 2008. « Evolutionary emergence of responsive and unresponsive personalities », *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 41, p. 15825–15830.

Young, A. J. & S. L. Monfort. 2009. « Stress and the costs of extra-territorial movement in a social carnivore », *Biology Letters*, vol. 5, no. 4, p. 439–441.

Young, K. M., S. L. Walker, C. Lanthier, W. T. Waddell, S. L. Monfort, & J. L. Brown. 2004. « Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses », *General and Comparative Endocrinology*, vol. 137, no. 2, p. 148–165.