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**RELATION HÉTÉROZYGOTIE-FITNESS  
CHEZ L'ÉPINOCHE À TROIS ÉPINES**  
**Des effets locaux substantiels non reflétés  
au niveau de l'effet global**

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## Résumé

La diversité génétique participe au potentiel évolutif des populations et des espèces. Sa conservation est un enjeu majeur dans la sauvegarde de la biodiversité. La diversité génétique peut aussi être importante pour l'individu. Cependant, il est souvent considéré que le niveau d'hétérozygotie a peu d'impact sur la fitness individuelle en nature. Toutefois, la relation entre l'hétérozygotie et la fitness a généralement été mesurée au niveau global (tout le génome), une approche potentiellement inadéquate. L'analyse de la relation hétérozygotie-fitness, à 30 marqueurs microsatellites et cinq indicateurs de fitness, dans une population sauvage d'épinoches à trois épines, révèle que des effets locaux de l'hétérozygotie importants et consistants ne se traduisent pas nécessairement en un effet global important, puisque l'effet à chaque marqueur génétique peut être opposé ou absent. Cette étude démontre l'importance de considérer les effets locaux de l'hétérozygotie pour obtenir une évaluation correcte de son impact dans les populations peu consanguines.

## Abstract

Conservation of genetic diversity is an essential goal of the biodiversity conservation challenge. Indeed, genetic diversity makes up the evolutionary potential of populations and species. Genetic diversity may also have significant consequences at the individual level. However, effects of heterozygosity on individual fitness are generally perceived as low in most populations. Nevertheless, the relationship between heterozygosity and fitness has usually been measured at the global level (genome wide), and this may be an inappropriate approach. Our analysis of the heterozygosity-fitness relationship in a wild population of threespine stickleback at 30 microsatellites loci and five fitness components revealed that important and consistent local effects of heterozygosity do not necessarily translate in an important global effect, since relationships at each genetic marker may be opposite in direction (positive versus negative) or nonexistent. Those findings show that, in non-inbred populations, heterozygosity must be considered at local level to accurately evaluate its impact.

## **Avant-Propos**

Au moment de conclure cette maîtrise je réalise l'immensité de l'expérience qu'elle a représentée pour moi. De l'immersion dans le monde de la biologie moléculaire, à la rencontre de dizaines de gens passionnés et passionnants, en passant par la participation à un colloque scientifique à *Sauðárkrúkur* (Islande), c'est enrichie que je ressors de ces années d'efforts, d'angoisses, mais aussi de plaisirs, d'apprentissage et de folie.

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En plus du travail mentionné ci-haut, j'ai également participé à l'étude du complexe majeur d'histocompatibilité chez l'épinoche à trois épines. Ces travaux sont maintenant poursuivis par Robin Martel et Scott McCairns et pourront faire l'objet d'une publication ultérieure.

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# **Chapitre 1**

## **Introduction générale**

## Conservation de la biodiversité

La biodiversité – ou diversité biologique – est le terme employé pour parler de la variété des formes de vie sur terre et de ses assemblages. Outre sa valeur intrinsèque, la diversité biologique fournit un nombre incalculable de services et de biens qui soutiennent la vie humaine (Secretariat of the Convention on Biological Diversity 2001). La conservation de la biodiversité est un des enjeux environnementaux le plus médiatisé. Cela, entre autre, grâce à la Convention de Rio de Janeiro sur la diversité biologique ratifiée par plus de 175 pays depuis 1992 (Secretariat of the Convention on Biological Diversity 2001), mais aussi, suite à l'extinction récente de plusieurs espèces et à la menace pesant sur de nombreuses autres (Hedrick 2001). Des milliers, voir des dizaines de milliers d'espèces, de même que des millions de populations uniques sont ainsi menacées d'extinction (Primack 2002). Cette extinction massive est le résultat des actions humaines (Primack 2002). En réponse, beaucoup d'efforts, de temps et d'argent sont investis dans l'espoir de conserver le maximum de biodiversité. Dans cette optique, il est essentiel de bien comprendre ce qu'est la biodiversité et quelle est son importance.

La biodiversité est souvent confondue avec le nombre d'espèces. Or, la biodiversité inclut, bien sûr, la diversité en terme de nombre d'espèces, mais aussi la diversité écosystémique et la diversité génétique intraspécifique (Secretariat of the Convention on Biological Diversity 2001; Templeton *et al.* 2001; Primack 2002). Il est essentiel de conserver la diversité génétique intraspécifique dans une optique de maintien à long terme de la biodiversité (Fraser and Bernatchez 2001; Hedrick 2001; Templeton *et al.* 2001; Primack 2002). En effet, la diversité génétique d'une espèce ou d'une population constitue son potentiel évolutif, ou potentiel à s'adapter à des changements environnementaux (Templeton *et al.* 2001; Frankham *et al.* 2002; Primack 2002; Reed *et al.* 2003). De plus, ce potentiel évolutif participe à la capacité de spéciation d'une population et donc, à la création de diversité en terme d'espèces, de communautés et d'écosystèmes (Templeton *et al.* 2001). La diversité génétique est un niveau particulièrement complexe de biodiversité, entre autre, parce que sa description demande l'emploi de techniques sophistiquées, mais aussi parce que son importance fait encore l'objet de litiges (Shields 1993; Caro and

Laurenson 1994; Caughley 1994; Keller and Weller 2002). Malgré ces difficultés, les analyses génétiques sont maintenant largement utilisées en conservation (Hedrick 2001; DeSalle and Amato 2004). Ces études en conservation génétique ont permis de constater que la diversité génétique intraspécifique a diminué suite à la réduction de la taille de nombreuses populations et ce, même au sein d'espèces apparemment saines (Primack 2002).

## Diversité génétique et hétérozygotie

La diversité génétique peut être mesurée de diverses façons, une de celles-ci consiste en l'évaluation du niveau de consanguinité. Ainsi, une des plus importantes et des plus anciennes contributions de la génétique à la conservation est la reconnaissance de l'importance du phénomène de dépression de consanguinité (Hedrick 2001). La dépression de consanguinité qualifie la diminution de la fitness reliée à l'augmentation du niveau de consanguinité des individus (Thornhill 1993). L'augmentation du niveau de consanguinité amène une perte d'hétérozygotie, c'est-à-dire une diminution de la proportion de gènes avec des allèles différents. Ceci entraînerait une diminution de la fitness principalement par l'expression d'allèles récessifs délétères ou encore par la perte de l'avantage de l'hétérozygote – ou phénomène de sur-dominance – (résumé dans Thornhill 1993). Le phénomène de consanguinité a aussi des répercussions sur la population. En effet, si elle est suffisamment répandue et soutenue, la consanguinité amène une diminution de la fitness moyenne de la population par accumulation de mutations délétères et par le phénomène de dépression de consanguinité (Keller et Weller 2002). Cet effet peut aller jusqu'à entraîner l'extinction de la population (Lynch *et al.* 1995; Frankham 1998; Keller et Weller 2002; pour un exemple, voir Saccheri *et al.* 1998). Minimiser la dépression de consanguinité devient ainsi un enjeu majeur pour la conservation de la diversité génétique et, à long terme, pour le maintien des populations et des espèces (Hedrick 2001; Wedekind 2002; Fiumera *et al.* 2004).

Les effets de la dépression de consanguinité ont traditionnellement été étudiés par l'analyse de la relation entre le niveau de consanguinité déterminé par le pedigree et une ou plusieurs composantes de la fitness (Thornhill 1993; Slate *et al.* 2004). Cependant, une

nouvelle méthode d'étude plus accessible s'est développée ces dernières années. Cette méthode s'appuie sur la cause du phénomène de dépression de consanguinité, c'est-à-dire la diminution du niveau d'hétérozygotie (ou augmentation de l'homozygote). Il s'agit, en effet, d'étudier directement la relation entre le niveau d'hétérozygotie et la fitness (David 1998; Hansson and Westerberg 2002; Slate *et al.* 2004). On parle alors de corrélation hétérozygotie-fitness (CHF). Le niveau d'hétérozygotie de chaque individu est déterminé par la proportion de marqueurs génétiques hétérozygotes. Au départ, les allozymes étaient utilisés comme marqueurs génétiques, mais ils font maintenant généralement place aux marqueurs neutres, principalement aux microsatellites (Hansson and Westerberg 2002; Pemberton 2004; Slate *et al.* 2004).

## **Importance biologique de la consanguinité et de l'hétérozygotie**

Malgré la reconnaissance populaire et scientifique de l'importance de la diversité génétique, une mésentente frappante existe au sein des biologistes sur l'importance du phénomène de dépression de consanguinité et de la corrélation hétérozygotie-fitness. On retrouve ainsi deux pôles d'opinion. D'une part, les biologistes s'intéressent aux questions de conservation ou de sélection sexuelle qui perçoivent souvent la dépression de consanguinité comme un phénomène majeur. D'autre part, les généticiens, particulièrement ceux qui étudient la dépression de consanguinité et la CHF en nature, qui l'évaluent souvent comme étant limitée, n'affectant de façon déterminante que les petites populations isolées et les populations avec un haut taux d'unions consanguines.

Par exemple, dans le cadre d'une revue de la littérature sur l'évitement de la consanguinité chez les animaux, Pusey et Wolf (1996) concluaient que la dépression de consanguinité est souvent suffisamment sévère pour entraîner l'évolution de comportement d'évitement de celle-ci. De plus, ils constatent que les comportements d'évitement de la reproduction consanguine sont très répandus, considérant leurs multiples formes; dispersion, copulations extra-couple et extra-groupe, choix du partenaire excluant les individus apparentés, maturité retardée, etc. (Pusey and Wolf 1996). Dans un même ordre d'idée, le maintien de la diversité génétique est une considération primaire dans les programmes de conservation, entre autre dans la reproduction assistée (e.g., Hedrick 2001;

(Wedekind 2002; Fiumera *et al.* 2004). Finalement, la dépression de consanguinité est généralement, et de plus en plus, considérée comme une force majeure dans l'évolution de la sélection sexuelle (e.g., Waser 1993; Mays and Hill 2004). Par exemple, elle pourrait être impliquée dans l'évolution de la polyandrie, du choix du partenaire pour des gènes de compatibilité et du choix cryptique de la femelle (e.g., Tregenza and Wedell 2002; Freeman-Gallant *et al.* 2003; Bretman *et al.* 2004).

Du côté des généticiens et des spécialistes de la CHF, la perception est généralement très différente. Ainsi, dans une revue de la littérature portant sur la relation hétérozygotie-fitness, David (1998) conclut que la CHF semble intrinsèquement faible et inconstante. Il note, en effet, que de nombreuses études ne détectent aucune relation ou même une relation négative. De plus, les CHF significatives sont généralement très faibles avec des coefficients de corrélation se situant habituellement entre 0.01 et 0.05 (David 1998). De façon similaire, Coltman et Slate (2003) concluent, suite à leur méta-analyse sur les études publiées et non-publiées de CHF, que la relation entre l'hétérozygotie et la fitness est différente de zéro, mais généralement faible ( $r^2$  moyen < 0.10). Il faut toutefois noter que la grande majorité des auteurs s'entendent sur l'existence du phénomène de dépression de consanguinité, le débat se situant plutôt sur sa portée et son ampleur (e.g., David 1998; Coltman and Slate 2003).

La corrélation hétérozygotie-fitness soulève également un autre débat. Il se rapporte à ce que l'hétérozygotie des marqueurs génétiques représente. Au départ, la CHF était considérée comme représentant le même phénomène que la dépression de consanguinité (Pemberton 2004). On considérait alors que l'hétérozygotie des marqueurs génétiques représentait l'hétérozygotie globale du génome, cette dernière résultant du niveau de consanguinité de l'individu (effet global) (David 1998; Hansson and Westerberg 2002). Toutefois, l'hétérozygotie des marqueurs génétiques pourrait plutôt uniquement se représenter elle-même (effet direct) ou encore représenter l'hétérozygotie à des gènes liés (effet local) (David 1998; Hansson and Westerberg 2002). Jusqu'à tout récemment, l'hypothèse de l'effet global était largement favorite (e.g., Coltman *et al.* 1998, 1999; Coulson *et al.* 1998, 1999; Saccheri *et al.* 1998; Marshall and Spalton 2000; Slate *et al.*, 2000; Höglund *et al.*, 2002; LeBas 2002; Foerster *et al.*, 2003; Grant *et al.* 2003).

Cependant, cette interprétation a été radicalement ébranlée par trois études récentes qui ont révélé que l'hétérozygotie moyenne, même mesurée à des dizaines de marqueurs génétiques, est un piètre estimateur du niveau de consanguinité de l'individu (Balloux *et al.* 2004; Markert *et al.* 2004; Slate *et al.* 2004; revue par Pemberton 2004).

Les travaux de Slate *et al.* (2004) et de Balloux *et al.* (2004) indiquent clairement que la relation entre l'hétérozygotie moyenne à des marqueurs génétiques et le niveau de consanguinité est forte seulement dans des situations extrêmes. En effet, les individus de la population doivent présenter une grande variation dans le niveau de consanguinité (Slate *et al.* 2004). Cela se produit dans les populations hautement structurées et dans les populations avec reproductions consanguines fréquentes (Balloux *et al.* 2004). Il semble donc qu'un phénomène autre que l'effet global de la consanguinité soit à l'origine de la plupart des CHF observées (Pemberton 2004). Puisque les marqueurs microsatellites sont généralement considérés neutres, l'hypothèse alternative la plus probable est celle des effets locaux. Les corrélations entre l'hétérozygotie aux marqueurs génétiques et la fitness résulteraient alors d'une association génétique entre un ou plusieurs des marqueurs et un ou des gènes effectifs (Hansson and Westerberg 2002).

## **Deux débats distincts ou le même débat ?**

Il est étonnant de constater la similitude entre le débat sur l'importance du phénomène de dépression de consanguinité et le débat sur les effets locaux versus globaux. Dans les deux cas, il s'agit de savoir si les effets de la consanguinité sont limités aux petites populations avec un phénomène de consanguinité particulièrement important. Le débat sur les effets locaux versus globaux a franchi un pas considérable grâce aux études de Balloux *et al.* (2004) et de Slate *et al.* (2004). Il semble maintenant évident que, dans la plupart des populations, les effets locaux expliquent mieux les CHF observées. Les effets globaux liés au niveau de consanguinité seraient donc réellement limités aux petites populations présentant beaucoup d'individus avec une consanguinité importante.

Il est révélateur d'appliquer ces résultats au débat sur l'importance de la consanguinité et de la CHF. Ainsi, si la dépression de consanguinité globale (affectant tout

le génome) n'est présente que dans les cas d'extrême consanguinité, il est possible que la faiblesse générale des CHF soit due à une méthode d'évaluation inadéquate. En effet, la vaste majorité des études sur la CHF ont été conduites dans une optique d'effet global. Conséquemment, l'importance de la CHF était déterminée par la corrélation entre l'hétérozygotie moyenne et la fitness. Or, les travaux de Balloux *et al.* (2004) et de Slate *et al.* (2004) indiquent que les CHF seraient généralement faiblement reliées à la consanguinité globale. Il serait donc généralement inadéquat de faire porter l'étude sur l'hétérozygotie moyenne.

En d'autres termes, il est adéquat de cumuler l'hétérozygotie des différents marqueurs lorsque l'on est en présence d'un effet global de la consanguinité, mais il est probablement inadéquat de le faire en présence d'effets locaux. En effet, sous l'hypothèse de l'effet global, l'hétérozygotie des marqueurs représente le niveau de consanguinité (Hansson and Westerberg 2002), il est alors pertinent de regrouper les différents marqueurs génétiques puisqu'ils représentent tous la même variable (David 1997). De surcroît, puisque la consanguinité a presque toujours un effet négatif sur la fitness (Charlesworth and Charlesworth 1987; Thornhill 1993), la CHF à chacun des marqueurs génétiques devrait être positive (l'hétérozygote ayant une plus grande fitness que l'homозигote). L'ensemble des CHF allant dans la même direction, il est possible de les additionner.

À l'opposé, lorsque la CHF est causée par des effets locaux, l'hétérozygotie à chacun des marqueurs peut avoir un effet positif sur la fitness, mais aussi un effet négatif ou aucun effet dépendamment de la fonction et des interactions alléliques aux gènes liés. C'est-à-dire que la CHF est potentiellement indépendante à chacun des marqueurs (David 1997). Dans cette optique, le cumul de l'hétérozygotie des différents marqueurs est susceptible d'entraîner une diminution dans la force de détection des CHF et de diminuer l'importance des relations. C'est pourquoi, nous croyons que l'importance de la corrélation hétérozygotie-fitness doit être mesurée dans un cadre d'analyse tenant compte de la possibilité d'observer des effets locaux.

## Objectifs et population à l'étude

L'objectif général de cette étude est donc d'évaluer l'importance des effets locaux de l'hétérozygotie dans une population sauvage d'épinoches à trois épines (*Gasterosteus aculeatus*). Après avoir vérifié que nous ne sommes pas en présence d'effets globaux grâce au test proposé par Balloux *et al.* (2004), nous évaluerons les effets locaux à 30 marqueurs microsatellites. L'effet de l'hétérozygotie sera évalué séparément pour chacun des marqueurs microsatellites et pour cinq composantes de la fitness : la survie, le succès d'appariement, la territorialité, la taille et la condition physique (résidus de la relation taille-masse). Ensuite, l'importance des CHF positives et négatives sera comparée. Finalement, l'effet global des CHF sera évalué pour fin de comparaisons.

L'épinuche à trois épines est un excellent modèle d'étude de la relation hétérozygotie-fitness pour plusieurs raisons. D'abord, plusieurs marqueurs microsatellites ont été décrits et positionnés sur une carte de liaison génétique pour cette espèce (Peichel *et al.* 2001). Ensuite, on retrouve, chez l'épinuche à trois épines, des comportements complexes incluant le choix du partenaire par la femelle, la compétition entre les mâles et la présence de soins parentaux prodigués par les mâles (Wootton 1976; Bell and Forster 1994; Bell 1995). On retrouve donc des bénéfices directs et indirects au choix du partenaire. De plus, le choix de la femelle est probablement de faible coût puisque les mâles se regroupent en forte densité et courtisent les femelles activement (Blais *et al.* 2004). De telles conditions sont à même de permettre l'évolution de comportements de choix du partenaire complexes, tel que la sélection de partenaire en fonction de gènes de compatibilité (Jennions and Petrie 1997). Finalement, les populations d'épinoches à trois épines présentent une grande diversité d'histoires évolutives (Bell and Forster 1994; Braithwaite and Odling-Smee 1999), ce qui devrait permettre d'en étudier les effets sur l'importance de la CHF dans le cadre d'études subséquentes.

Concrètement, les objectifs spécifiques de l'étude sont : (i) de tester l'occurrence d'effets locaux de l'hétérozygotie à 30 marqueurs microsatellites sur cinq composantes de la fitness dans une population sauvage d'épinoches à trois épines, (ii) d'évaluer l'importance de l'impact sur la fitness de ces CHF locales, (iii) de comparer les résultats obtenus par l'analyse des effets locaux aux résultats obtenus par une analyse de type effet

global. Finalement, les conséquences évolutives potentielles des CHF à effets locaux seront discutées de même que la signification des résultats dans un contexte de conservation de la biodiversité.

# **Chapitre 2**

## **Local heterozygosity-fitness correlations with global positive effects on fitness in threespine stickleback**

Mélissa Lieutenant-Gosselin, Louis Bernatchez

## Résumé

Une relation positive entre l'hétérozygotie moyenne à des marqueurs génétiques et la fitness a été décrite fréquemment et interprétée comme de la dépression de consanguinité (hypothèse d'effet global). Cependant, des études récentes ont démontré la faiblesse générale du lien hétérozygotie-consanguinité. Conséquemment, l'hypothèse d'une relation résultant de la liaison génétique locale entre marqueurs et gènes effectifs (hypothèse des effets locaux) a gagné en crédibilité. L'importance et l'impact évolutif des relations hétérozygotie-fitness locales ont rarement été évalués. Les effets locaux de l'hétérozygotie ont été mesuré dans une population sauvage d'épinches à trois épines. Trente marqueurs microsatellites ont été étudiés en relation avec cinq traits de fitness. Des corrélations hétérozygotie-fitness locales hautement significatives et temporellement stables ont été observées. Les effets locaux de l'hétérozygotie étaient principalement positifs, cependant des effets négatifs étaient aussi présents. Parallèlement, l'effet global de l'hétérozygotie était généralement faible. Des effets locaux d'importance similaire sont probablement fréquents, mais non détectés dans les études d'effet global de l'hétérozygotie.

## Abstract

The complex interactions between genetic diversity and evolution have important implications in many biological areas including conservation, speciation and mate choice. A common way to study these interactions is to look at heterozygosity-fitness correlations (HFCs). Until recently, HFCs based on non-coding markers were believed to result primarily from a global inbreeding effect. However, accumulating theoretical and empirical evidence is showing that HFCs may often result from genes being linked to the markers used (local effect). Moreover, local effect HFCs could differ from a global inbreeding effect in their direction and occurrence. Consequently, the investigation of the structure and consequences of local HFCs is emerging as a new important goal in evolutionary biology. In this study of a wild threespine stickleback (*Gasterosteus aculeatus*) population, we first tested the presence of significant positive or negative local effects of heterozygosity at 30 microsatellite loci on five fitness components: survival, mating success, territoriality, length and body condition. Then, we evaluated the direction and shape of total impact of local HFCs, and estimated the magnitude of the impacts on fitness using regression coefficients and selection differentials. We found that multi-locus heterozygosity was not a reliable estimator of individual inbreeding coefficient, which supported the relevance of single-locus based analyses. Highly significant and consistent local HFCs were observed. These HFCs were mainly positive, but negative effects of heterozygosity were also found. Strong and opposite effects of heterozygosity are probably present in many populations, but may be blurred in HFC analyses only looking for global effects. In this population, both negative and positive HFCs are apparently driving mate preference by females. Such a pattern of mate choice is likely to contribute to the maintenance of both additive and non-additive genetic variance.

## Introduction

The interaction between genetic diversity and fitness is a key element in evolutionary biology, and particularly so for studies related to the evolution of mate choice, dispersal, host-pathogen interactions, as well as speciation (reviewed in Charlesworth and Charlesworth 1987; Thornhill 1993; Lynch et al. 1995; Hedrick and Kalinowski 2000; Keller and Waller 2002). The importance of understanding such processes for conservation biology is also well acknowledged (DeSalle and Amato 2004). Elucidating the structure and dynamics of genetic diversity-fitness interactions may allow the evaluation of the relative importance of local adaptation versus evolutionary potential of populations in a conservation context (Vergeer et al. 2004). This issue is also intimately related to testing the alternate hypotheses of good genes versus complementary genes in studies of mate choice (Mays and Hill 2004; Neff and Pitcher 2005).

A common way of studying the dynamics of genetic diversity-fitness interactions has been to investigate inbreeding depression and its possible evolutionary consequences (e.g., Charlesworth and Charlesworth 1987; Thornhill 1993; Coltman and Slate 2003). The level of inbreeding has traditionally been determined by pedigree analysis, but is increasingly documented by means of quantifying heterozygosity using either allozymes or non-coding markers such as microsatellites (Slate et al. 2004). For non-coding markers, it has generally been assumed that mean heterozygosity reflects a global level of heterozygosity, which in turn should correlate with individual inbreeding level (e.g., Coltman et al. 1998, 1999; Coulson et al. 1998, 1999; Saccheri et al. 1998; Marshall and Spalton 2000; Slate et al. 2000; Höglund et al. 2002; LeBas 2002; Foerster et al. 2003; Grant et al. 2003).

This interpretation was radically challenged by three recent studies that revealed a weak association between heterozygosity, even when measured over many markers, and inbreeding coefficient (Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004; *reviewed in* Pemberton 2004). According to Slate et al. (2004), the relationship between heterozygosity and inbreeding is expected to be strong only in the case of unusually high variance in individuals' inbreeding coefficients. Similarly, using stochastic individual-

based simulations, Balloux et al. (2004) found a high correlation between heterozygosity and inbreeding only in highly sub-structured populations or populations with mating systems favouring frequent incestuous pairings. Yet, it is unlikely that the numerous empirical studies in which heterozygosity-fitness correlations (HFCs) were detected all belong to such extreme situations (Pemberton 2004). Altogether, these observations suggest that either direct or local effects may explain HFC better than global inbreeding depression (Pemberton 2004).

The difference between direct, local, and global inbreeding effects stands in what is being characterized by genetic markers. In global inbreeding effects, marker heterozygosity is expected to reflect genome wide heterozygosity, which should correlate with individual inbreeding level. In direct effects, markers heterozygosity represents only their own heterozygosity, whereas they represent linked loci heterozygosity in local effects. Direct effects are unlikely in the case of non-coding markers (Hansson and Westerberg 2002). Therefore, the most likely alternative hypothesis to global inbreeding effects is that of local effects, which should primarily result from a genetic association, through linkage disequilibrium, between effective genes and one or more of the markers used (Hansson and Westerberg 2002).

Arguably, considering HFC from a local rather than global inbreeding perspective reorients several basic questions and goals of HFC studies. First, since the importance of local HFCs is not resolved (Pemberton 2004), the proportion and number of loci presenting local HFCs, and the importance of their effects on fitness still have to be investigated. Several studies recently provided evidence for HFCs associated with local effects (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005). However, most of these studies included relatively small numbers of loci with unknown chromosomal positioning, which limits the interpretation of the results obtained (Pemberton 2004). A second important aspect of local effects concerns the possibility of observing both negative and positive HFCs. Thus, under the inbreeding hypothesis, heterozygosity at one locus is expected to represent global inbreeding level (Hansson and Westerberg 2002). As inbreeding generally affects fitness negatively (Charlesworth and Charlesworth 1987; Thornhill 1993), heterozygosity at every locus is expected to positively

correlated with fitness. In the case of local effects, however, heterozygosity at a given locus could have an independent effect on fitness components (David 1997), either positive, negative or null, depending on the functional importance and allelic interactions (e.g., additivity versus dominance or overdominance) at linked genes. One way to approach this issue is to first test whether heterozygosity at each locus has a positive or negative effect on fitness, and then to assess the global relationship over all local HFCs. These are two important issues as the relative importance of both types of effects could dictate the direction of evolution in terms of the maintenance of genetic diversity under the effect of either directional or stabilizing selection.

The general objective of this study was to investigate the occurrence and magnitude of local effect HFC in a wild population of threespine stickleback (*Gasterosteus aculeatus*) from the St. Lawrence River estuary, Québec, Canada. The threespine stickleback represents a remarkable model for studying HFC in natural conditions. First, many microsatellite loci have been described and positioned on a QTL linkage map (Peichel et al. 2001). Second, stickleback populations have apparently evolved under different selective pressures, both in terms of directionality and intensity, such that the species comprises highly specialized, as well as more generalist populations (Bell and Forster 1994; Braithwaite and Odling-Smee 1999). The species therefore offers the potential to evaluate the nature of HFC under diverse population histories and selective regimes. Third, the threespine stickleback is also characterized by complex behavioral interactions, including female mate choice and male-male competition, as well as paternal care (Woottton 1976; Bell and Forster 1994; Bell 1995). Therefore, mate choice may lead to both direct and indirect benefits to females a situation likely to increase the possibility of complex mate choice evolution, such as mating based on genetic compatibility (Jennions and Petrie 1997). Moreover, intense male-male competition could inflate fitness differences between more or less heterozygous individuals, as reported for other types of stress (e.g., Danzmann et al. 1988; Borsa et al. 1992; Audo and Diehl 1995).

In this context, our specific objectives were: (i) to test for the occurrence of significant positive or negative local effects of heterozygosity at 30 microsatellite loci evenly distributed over the genome, on five fitness components; (ii) to evaluate the

direction and shape of the total impact of local HFCs; (iii) to estimate the magnitude of the impact on fitness by means of both regression coefficients and selection differentials. Finally, we discuss possible evolutionary consequences of local HFCs.

## **Materials and methods**

### **Study site and field sampling**

We studied a natural population of threespine stickleback located at the Isle-Verte Ecological Reserve on the south shore of the St. Lawrence River middle estuary (Québec, Canada). Sticklebacks of this population live one to two years (De Fraipont et al. 1993) and most males complete only one breeding cycle per year (Lachance and FitzGerald 1992). They reproduce in coastal tide pools reached during spring high tides (Blais et al. 2004).

Fish were collected in May after the egg-collecting phase of the first breeding cycle (detailed in Blais et al. 2004). Virtually all males and the majority of females from five interconnected tide pools were captured. A total of 605 adult fish were captured including 325 (54%) females and 280 males. Approximately half the males were guarding a nest (139) and 35% of the nests had eggs (Blais et al. 2004). In this population, non-territorial sneakers achieve virtually null reproductive success. Thus, non-territorial males, which represented nearly 50% of all males in the pools, fertilised only 3% of the eggs (Blais et al. 2004). A total of 208 males were available for genotyping, including most territorial ( $n=135$ ) and 73 randomly selected non-territorial ones. Sixty percent of these males were 1+ and 40% were 2+ age classes (Blais et al. 2004).

### **Fitness components**

Five male fitness related traits were measured: survival, standard length, body condition, territoriality status (having a nest or not) and mating success (detailed in Blais et al. 2004). The two cohorts were analysed separately except for survival (see below). Standard length (length from the tip of the snout to the posterior extremity of the hypurals) at a given age was used as a surrogate for growth. Blais et al. (2004) previously found a highly significant effect of this trait on the probability of being a territorial male for 1+ males. Body

condition was defined as the residual of the cohort linear regression predicting body weight from standard length using JMP IN 3.2.6 (SAS Institute 1999), a simple and straightforward approach. Territoriality relates to male-male competition and was defined as the possession (or lack thereof) of a nest, and was determined by visual observations of the males' behaviour in the field (detailed in Blais et al. 2004). Mating success of territorial males ( $n = 77$  males 1+,  $n = 58$  males 2+) was defined as successful (egg clutch(es) present in their nest) or unsuccessful (no eggs). These five fitness components were included since they are relatively independent from each other (Table 1). Finally, to assess the possibility of differential survival as a function of genetic diversity, we tested the null hypothesis of no difference in distribution of heterozygosity values in fish from different age classes.

### **Genetic analyses**

A total of 30 microsatellites were chosen among 209 loci identified by Peichel et al. (2001) in order to cover half of the 26 identified linkage groups (Table 2). Seven of those loci are linked to QTL associated either with the number of lateral plates, length of first or second dorsal spine, or gill raker numbers (Peichel et al. 2001). Lateral plates and dorsal spines play a role in defence against bird and fish predators, while gill raker numbers is involved in prey selectivity (Bell and Foster 1994).

Polymerisation chain reactions (PCRs) were carried out on individual locus in a 11- $\mu\text{L}$  reaction containing: 2  $\mu\text{L}$  (25-50 ng) of total genomic DNA, 1.2  $\mu\text{L}$  of reaction buffer (10.9 mm Tris-HCL, ph 9.0, 54.5 mm KCl, 1.6 mm MgCl<sub>2</sub>, 0.1% Triton X-100), 56.8  $\mu\text{M}$  of dGTP, dCTP and dATP, 28.4  $\mu\text{M}$  of dTTP, 0.5 pmol of forward and reverse primers, 1.7  $\mu\text{M}$  of dUTP TAMRA (Molecular Probe) fluorescent incorporation labelling and 1 unit of *Taq* DNA polymerase. PCRs were conducted in a Perkin-Elmer 9600 thermocycler (version 2.01) with an initial denaturation step of 5 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 1 min at appropriate annealing temperature (Table 2) and 1 min at 72 °C, ending with a 10 min of elongation phase at 72 °C. PCR products were electrophorized on 8% denaturating polyacrylamide gels, allelic bands were visualized on a FMBIO II scanner (Hitachi) and scored using the GENESCAN-500 size standard (Applied Biosystems Inc.).

### Single-locus HFC analyses

Expected and observed heterozygosities were calculated using GENETIX, version 4.01 (Belkhir et al. 1996-2002), and departure from Hardy-Weinberg equilibrium (HWE) and  $F_{is}$  estimates were calculated using GENEPOP version 3.1d (Raymond and Rousset 1995). The relationship between departure from HWE and presence of significant HFC was tested by a  $\chi^2$  test and the effect of allele number and expected heterozygosity on departure from HWE were tested using logistic regressions in JMP IN software (SAS Institute 1999).

Prior to analysing single-locus HFCs, we first tested for evidence of global inbreeding effect. Under the assumption that heterozygosity reflects individual inbreeding level, theory predicts that heterozygosity of loci within an individual should be correlated (Balloux et al. 2004; Pemberton 2004). This can be verified by randomly subdividing the sample of loci into two groups and asking whether the mean multi-locus heterozygosity (MLH) of the first group of loci is correlated with the MLH of the second group of loci within individuals (Balloux et al. 2004). The MLH-MLH correlation coefficient is then interpreted as an indication of the magnitude of the association between MLH and inbreeding coefficient (Balloux et al. 2004). We randomly subdivided loci into two groups of 15, recalculated individual MLH for both groups and measured the correlation between these measures with a linear regression. This was repeated 10,000 times using MAPLE release 7 (Waterloo Maple Inc.) to obtain the mean and standard deviation of the correlation coefficient.

The occurrence of positive or negative effects of single-locus heterozygosity on fitness components was first investigated by  $\chi^2$  tests in the case of binary components (survival, territoriality, mating success) and two-tailed *t*-tests for continuous traits (standard length, body condition) using JMP IN software (SAS Institute 1999). Correction for multiple tests was applied by calculating the number of false-positive associations expected at  $\alpha = 0.05$  for 30 independent tests (one per locus). For each component and each age class, 1.5 false-positive associations were expected or 13.5 in total (9 components-age class x 1.5). Hence, the presence of real associations was tested by a  $\chi^2$  comparing the observed number of significant associations ( $P \leq 0.05$ ) with the expected number of false positive (13.5) using SAS release 8.02 (SAS Institute Inc.).

Secondly, the predominance of negative versus positive single-locus HFCs was investigated by a cumulative binomial exact probability test on the direction of the associations (using SAS release 8.02, SAS Institute Inc.). For example, suppose that 20 of the 30 loci revealed a positive association between heterozygosity and a given fitness component, and 10 revealed a negative association. The test calculated the probability of obtaining  $\geq 20$  positive relationships on 30 trials assuming equal probabilities for one result or another (50%), and independently of the absolute level of significance of single-locus HFCs. Also, in order to investigate the stability of the response among fitness components and age cohorts (temporal stability), we performed a categorical model analysis using the CATMOD procedure in SAS release 8.02 (SAS Institute Inc.). This procedure determines the importance of each independent variable (fitness component and age cohort) in explaining the variance observed in the response variable (direction of the effect of heterozygosity; positive or negative).

Thirdly, we also tested whether the combination of loci falling into one of the two categories (positive or negative single-locus HFC) had a significant impact on each fitness component. This was achieved using the Fisher method combining probabilities of independent tests (Sokal and Rohlf 1995), as employed by Bierne et al. (2000) in an analogous context.

Finally, we determined whether the distribution of single-locus HFCs was random among loci by performing a  $\chi^2$  comparing the distribution of the observed associations versus the expected uniform distribution for all components and both age classes using SAS release 8.02 (SAS Institute Inc.).

### **Estimating the total impact of local HFCs**

To infer the possible evolutionary consequences of the observed pattern of local HFCs, we explored the shape and strength of relationships between each fitness component and global heterozygosity resulting from the cumulative effect of local associations. Global heterozygosity was estimated by multilocus heterozygosity (MLH) that is the proportion of heterozygote loci over all loci for each individual. The regressions of fitness traits on MLH were then examined graphically to detect any non-linear relationships, as performed by

Amos et al. (2001) (see also Neff 2004 for an example of non-linear HFC). The significance of the best-fit model was tested using JMP IN software (SAS Institute 1999). The strength of global impacts was also evaluated by calculating selection differentials. For both continuous components (standard length and body condition), we used the method of Lande and Arnold (1983) whereby the selection differential may be inferred from the slope of the regression between standardised MLH and relative fitness component. In the case of fitness components with a binary response (survival, mating success and territoriality), we calculated standardized selection differentials according to Janzen and Stern (1998), which is an adaptation of Lande and Arnold (1983) method for using a logistic regression. We performed this analysis separately for both groups of loci showing evidence for either positive or negative HFC.

## Results

### Descriptive genetic parameters and MLH-inbreeding correlation

The mean number of alleles per locus was 14.3, and ranged from 2 to 30 (Table 2).  $H_o$  were on average slightly below  $H_e$  ( $H_o = 0.67 \pm 0.17$ ;  $H_e = 0.74 \pm 0.19$ ), and 16 loci revealed significant departure from HWE, which resulted in a mean  $F_{is}$  of  $0.075 \pm 0.068$  (Table 2). Departure from HWE was associated with allelic richness at a given locus (Logistic regression,  $\chi^2 = 6.24$ ,  $P = 0.013$ ,  $df = 1$ ,  $n = 30$ ,  $r^2 = 0.15$ ), and to single-locus  $H_e$  as well (Logistic regression,  $\chi^2 = 8.21$ ,  $P = 0.004$ ,  $df = 1$ ,  $n = 30$ ,  $r^2 = 0.20$ ). However, the departure from HWE had no significant effect on the probability of loci to show significant HFC ( $\chi^2 = 0.00$ ,  $P = 0.961$ ,  $df = 1$ ). The correlation coefficient between MLH computed with the randomly created subsets of 15 loci was weak (mean  $r^2 = 5.5\% \pm 1.9\%$ , 10,000 permutations). This indicated that MLH was not a reliable estimator of individual inbreeding coefficient, and confirmed the relevance of subsequent single-locus based analyses.

## Single-locus HFCs

### *Occurrence of positive and negative single-locus HFCs*

Between zero and six significant single-locus HFCs (mean =  $2.78 \pm 1.56$ ) were observed depending on the fitness component and the age class analysed. In total, 25 of the 270 single-locus HFCs were significant, which represented a highly significant difference from the expected false discovery rate of 13.5 ( $\chi^2 = 10.31, P = 0.001, \text{df} = 1$ ). Eighteen of the 25 significant HFC were positive, and seven were negative. Considering all single locus HFCs independently of their level of significance, positive relationships represented 63% of all associations (171/270), a highly significant difference from random expectation (cumulative binomial probability test,  $P < 0.001$ , trials = 270). When considering fitness components separately, a significant predominance of positive HFCs was observed for survival and territoriality at both ages (Fig. 1). Positive, albeit non-significant, single-locus HFCs were predominantly observed for standard length and body condition as well. In contrast, similar (for 1+ fish) or larger numbers (for 2+ fish) of negative HFCs were observed for male mating success (Fig. 1). Furthermore, the categorical model analysis indicated that proportions of positive versus negative single-locus HFCs significantly varied among fitness components, and were temporally stable between cohorts (Table 3).

### *Overall significance of single-locus HFCs*

Fisher's method for assessing the overall significance of multiple tests was applied separately for loci showing either positive or negative effects (Table 4). The overall effect of loci showing a positive association between heterozygosity and fitness was significant for all fitness components in at least one age class (Table 4). Moreover, the overall effect of loci showing a negative association was highly significant for mating success for both age classes (Table 4). No overall significant effect was observed between loci with a negative association and any other trait.

### *Non-random HFCs distribution*

The observed distribution of significant single-locus HFCs seems to be non-random ( $\chi^2 = 40.56, P = 0.069, \text{df} = 29$ ). Thus, three loci (*Stn34*, *Stn78*, *Stn83*) were associated with

three different fitness components, and six other loci (*Stn9*, *Stn31*, *Stn107*, *Stn130*, *Stn147*, *Stn177*) revealed two significant associations, the remaining four being associated to a single component (*Stn14*, *Stn23*, *Stn26*, *Stn96*). These 13 loci were distributed over nine linkage groups and included loci associated with the length of both the first (*Stn9*, *Stn26*), and second dorsal spines (*Stn96*, *Stn130*) (Table 2).

### **Global impact of single-locus HFCs**

To infer the possible evolutionary consequences of the observed pattern of local HFCs, and also for the sake of comparison with previous studies that focussed on global effects only, we explored the shape and strength of relationships between each fitness component and global heterozygosity resulting from the cumulative effect of local associations. MLH over all 30 loci ranged from 0.38 to 0.90, with an average of  $0.66 \pm 0.11$ , which explained an average of 5% of the variance in fitness components, and ranged from 3 to 9%, depending upon the trait for which a significant association was observed. A significant positive relationship was observed between MLH calculated for 30 loci and survival (Logistic regression,  $\chi^2 = 9.41$ ,  $P = 0.002$ ,  $df = 1$ ,  $n = 208$ ,  $r^2 = 0.03$ ), territoriality in both age groups (Logistic regressions, 1+:  $\chi^2 = 4.98$ ,  $P = 0.026$ ,  $df = 1$ ,  $n = 125$ ,  $r^2 = 0.03$ ; 2+:  $\chi^2 = 9.46$ ,  $P = 0.002$ ,  $df = 1$ ,  $n = 83$ ,  $r^2 = 0.09$ ) and standard length for 1+ fish (Fig. 2), whereas similar, non-significant trends were observed for 2+ males in standard length, and for 1+ males in the case of body condition (Fig. 2). No apparent relationship with MLH was detected with mating success in either age class, nor with body condition in 2+ males (Figs. 2 and 3). In all cases where a relationship was observed between MLH at the 30 loci and a fitness trait, the best fit was obtained by positive linear relationship (Figs. 2 and 3).

### *Selection differentials*

Selection differentials for each fitness related trait were calculated for the effects of MLH calculated over all 30 loci, as well as for loci with either positive or negative HFC relationships (Table 5). Selection differentials ( $S$ ) calculated with MLH (30 loci) were all positive except for mating success in 2+ fish (-0.12 SE 0.17). The average  $S$  value was 0.06, with the highest value being observed for survival (0.26 SE 0.09) and 2+ territoriality (0.21 SE 0.08). Selection differentials calculated with loci showing positive HFC were

globally higher with an average effect of 0.24 and ranged from 0.01 SE 0.00 (2+ length) to 0.64 SE 0.20 (2+ mating success) (Table 5).  $S$  acting upon length and body condition were consistently lower, possibly because of the small variance in these traits (data not shown). Finally,  $S$  calculated from MLH restricted to negative single-locus HFCs for mating success were -0.46 SE 0.15 in 1+ males, and -0.59 SE 0.17 in 2+ males (Table 5).

## Discussion

Balloux et al. (2004), and Slate et al. (2004) have both shown the inadequacy of considering a priori HFCs as a manifestation of global inbreeding depression. As a result, their findings have reinforced the notion of heterozygosity effects on fitness at a local level, consistent with recent empirical studies finding support for local effect HFCs (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005). Yet, doubts persist around the existence and importance of local effect HFCs in natural populations (Pemberton 2004). In this regard, our aim was to investigate the presence, characteristics, magnitude and consequences of local heterozygosity effects in a natural population of threespine sticklebacks.

### **Strong evidence of positive and negative single-locus HFCs**

Significant local HFCs were observed in all fitness components: survival, mating success, territoriality, standard length and body condition. Together with the very weak observed correlation of heterozygosity among loci, these results confirmed that HFC could arise without global inbreeding effects (Balloux et al. 2004). In contrast to global effect HFCs, the direction of local effect HFCs cannot be easily predicted. However, an intuitive hypothesis is to expect complex interactions between null, positive and negative HFCs depending on the loci and the traits under study. In this population, heterozygosity mainly had a positive effect on fitness, while positive single-locus HFCs were predominant in all fitness components except mating success, and showed a temporal stability between cohorts. In fact, mating success presented all but one significant negative single-locus HFCs that were detected. Moreover Fisher test revealed significant grouped positive effects for all components, whereas grouped negative effects were significant only with

respect to mating success. Furthermore, heterozygosity at some of the loci had apparently no impact at all on fitness for the traits we studied. Thus, heterozygosity-fitness correlations are more complex for local effects compared to those generally reported for global inbreeding effects.

### Possible evolutionary consequences

Our results indicated that males that are more heterozygous at certain loci have a higher fitness because: (i) they are generally larger; (ii) they have a higher probability of establishing a territory; (iii) territorial males obtain the vast majority of fertilizations (Blais et al. 2004); (iv) among territorial males, heterozygous individuals (at some loci) are more likely to reproduce and finally; (v) heterozygous males are more likely to live two years, and therefore, potentially reproduce more than once (Lachance and FitzGerald 1992). Thus, even if some of the fitness components used may not be correlated with total fitness (Hunt et al. 2004), the use of many components (Conner and Hartl 2004) and the detection of effects for all of them reveals an unequivocal effect of heterozygosity on fitness. Moreover, the impacts of heterozygosity on fitness were relatively strong in many cases. For instance, heterozygosity at 11 to 21 loci explained on average 13% (and up to 22%) of the variance for fitness components. Furthermore, heterozygosity at those loci generated high selection differentials by changing males' survival, territoriality and mating success probabilities. For example, the selection differential from positive HFCs was 0.36 SE 0.09 for males' survival, 0.56 SE 0.17 for mating success in 1+ males and 0.64 SE 0.20 in 2+ males. These values are within the range, yet substantially higher than the median value of 0.16 reported by Kingslover et al. (2001) in a recent meta-analysis.

Our finding of substantial heterozygosity impacts on fitness contrasts with the small effects usually reported in studies of inbreeding depression on natural animal populations, studies which have led to the general conclusion of weak impacts of heterozygosity on fitness (David 1998; Hasson and Westerberg 2002; Slate and Pemberton 2002; Coltman and Slate 2003). The difference between most previous studies and our stems primarily from differences in analytical approaches, rather than the specificity of the focal population. Thus, we believe that emphasis on local effects may be more efficient at deciphering the complexity of interactions between heterozygosity and fitness. For

example, it allowed quantifying the differential impacts of loci with negative and positive effects separately. Such effects are likely to be hidden in analyses focussed on global effects only. Indeed, strong impacts of heterozygosity on fitness associated with local effects are not so surprising when considering the emerging picture of consistent, non-additive genetic effects on phenotypic expression, including dominance and overdominance (e.g., Meffert et al. 2002; Sgrò and Blows 2003; Gibson et al. 2004; Syed and Chen 2005; see also Neff and Pitcher 2005).

Different evolutionary outcomes could arise from heterozygosity effects on fitness. For instance, it is probable that, at loci showing positive HFCs, alleles will persist longer in the population as a result of balancing selection. Moreover, positive HFC could increase positive selection upon new mutations, and together, these two effects could lead to maintenance of genetic diversity within the population (Balloux et al. 2004). Interestingly, loci showing at least one significant positive HFC tended to have slightly more alleles (Table 2), and included none of the seven loci having fewer than 10 alleles. Hansson et al. (2004) found similar results in a HFC study on great reed warblers. In their study, the strongest single-locus positive HFCs were found in the four markers (out of 19) showing the highest heterozygosity. Positive HFCs could also promote dispersal through an increase of migrant indirect fitness because their offspring are more likely to be heterozygous compared to those of local parents. This would be analogous to theoretical expectations in the case of inbreeding depression (Thornhill 1993).

### **Possible evolutionary consequences on mate choice**

HFC may also have biologically meaningful impacts in the evolution of mate choice. Mate choice by female threespine sticklebacks could have both direct (material) and indirect (genetic) benefits, since males not only provide females with sperm, but also aerate and defend eggs and fry against predators (Wootton 1976). Therefore, the observed general positive single-locus HFCs may promote the evolution of two nonexclusive types of mate choice: females could either choose a more heterozygous male to increase direct benefits (e.g., more heterozygous males have a better survival), or a compatible male (e.g., one having different alleles at effective loci) in order to increase offspring quality (indirect benefits). Finally, HFC could also contribute to the evolution of polyandry, since multiple

mating has been shown to result in higher genetic diversity and progeny survival (e.g., Garant et al. 2005). However, polyandry is a limited phenomenon in this population (Whoriskey et al. 1986; Bell and Forster 1995; Blais et al. 2004).

A very interesting result stemming from this study is the finding that both important positive and negative single-locus HFCs affect males' mating success. Single-loci showing positive and those showing negative effects could either influence the same trait or different traits used by females in mate choice. Given that heterozygosity had a positive effect for other fitness components, it is plausible that negative and positive HFCs may affect different traits. An attractive hypothesis, therefore, is that negative HFCs might influence male ornaments under direct selection, while positive HFCs could affect general male quality. Traits affected by either negative or positive single-locus HFCs could thus be integrated through multiple cues in female mate choice, whereby females choose both heterozygous males at random loci (good fathers; direct benefit) and for indirect benefits by choosing individuals homozygous at particular good genes. Finally, genetic compatibility (e.g., in terms of dissimilarity) could represent another cue that could be integrated in a hierarchical scheme for choosing mates, as demonstrated empirically by Roberts and Gosling (2003) and reviewed in Mays and Hill (2004).

Recently, Neff and Pitcher (2005) proposed to link 'good genes' with additive genetic variation in fitness and 'compatibility genes' with non-additive genetic variation in fitness. In their perspective, genetic quality becomes the sum of additive and non-additive quality (Neff and Pitcher 2005). Thus, mate choice may (i) oscillate between a good genes mating system and compatibility genes mating system, (ii) rely on both good genes and compatibility genes in the same female simultaneously, or (iii) rely on both good genes and compatibility genes in different females with negative frequency dependent selection on the two female types (Neff and Pitcher 2005). Such a model for mate choice is appealing, as it allows the maintenance of both additive and non-additive genetic variance and thus helps to elucidate an important evolutionary paradox (Neff and Pitcher 2005; see for example Roff and Mousseau 1987). Although not considered by Neff and Pitcher (2005), this model could be expanded to consider mate choice for direct benefits. For instance, here we found that females chose males that were more heterozygous at several loci. This

behavior is likely to provide females with better fathers, and also to favor maintenance of both additive and non-additive variance. Indeed, even if females choose consistently for a non-additive male quality, this quality is not heritable per se, and thus will not respond to selection by a decrease in variability.

### **Generality of the findings**

In addition to this study, several recent papers provided evidence for the importance of local associative effects in explaining HFC in different species and contexts (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005). It thus appears that single-locus HFCs may be more common than previously assumed. In this study, however, the local characteristics of the Isle-Verte's stickleback population may have increased the prevalence of single-locus HFCs. For example, this population is located within the transition between fresh and salt water, which might present a particularly heterogeneous environment prone to heterozygote advantage. Also, its effective population size is likely larger than small isolated populations, and therefore less likely to be purged of recessive deleterious alleles (Lande and Schemske 1985). Moreover, intense male-male competition in this population (Blais et al. 2004) could contribute to inflate fitness differences between more or less heterozygous individuals.

### **Concluding remarks and future directions**

Our study revealed the occurrence of important and temporally stable local HFCs in a natural population of threespine sticklebacks. These HFCs are mainly positive, but negative effects of heterozygosity also exist. Strong and opposite effects of heterozygosity are probably present in many populations, but may have been blurred in HFC studies based on a global effect perspective only. In this population, both negative and positive HFCs are apparently driving mate preference by females. This type of mate choice is likely to contribute to the maintenance of both additive and non-additive genetic variance. The use of non-coding markers has made the exploration of HFC easier by providing numerous heterozygous loci, but does not allow the identification of effective genes or alleles. However, the repartition of the single-locus associations tended to be non-random among loci, which could indicate that heterozygosity at certain loci may affect more than one

fitness component. Based on the results of several recent studies, including ours, we propose that the time may be ready to switch from the local versus global effects debate to questions that may bring more light on the evolutionary significance of HFC, such as: (i) the identification of the effective genes underlying HFC; (ii) the evaluation of the mechanisms creating HFCs, such as dominance, overdominance and genotype-by-environment interactions; (iii) the determination of the relative importance of additive and non-additive aspects of genetic quality and their interactions, and; (iv) the evaluation of the impacts of different evolutionary histories and environmental factors on the expression of HFC.

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**TABLE 1. Correlations among fitness components as estimated by the coefficient of determination ( $r^2$ ).** Results for 1+ males are shown in a) and results for 2+ males in b).

a)	Survival	Territoriality	Mating success	Standard length	Body condition
Survival	-				
Territoriality	0\$	-			
Mating success	0	0	-		
Standard length	0	0.20	0.02	-	
Body condition	0	0.00	0.00	0.00	-

b)	Survival	Territoriality	Mating success	Standard length	Body condition
Survival	-				
Territoriality	0	-			
Mating success	0	0	-		
Standard length	0	0.00	0.01	-	
Body condition	0	0.03	0.00	0.00	-

\$ In the case of survival and territoriality, a “0” without decimals indicates that the correlation could not be estimated as fish from different age groups or territoriality status were separated for these fitness components. In contrast, a “0.00” indicates a calculated coefficient of determination.

**TABLE 2. Descriptive statistics and PCR annealing temperatures for the 30 loci genotyped.** Linkage groups, and QTL are those defined by Peichel et al. (2001); A, number of alleles;  $H_o$ , observed heterozygosity;  $H_e$ , expected heterozygosity;  $F_{is}$ , average fixation index of individuals relative to sub-populations according to Robertson and Hill (1984). Loci with significant single-locus HFC for at least one fitness component are shown in bold characters.

Linkage		QTL	A	$H_o$	$H_e$	$F_{is}$	Annealing	
Locus	group						T (°C)	
<b><i>Stn1</i></b>	I	-	16	0.67	0.85	0.185*	60	
<b><i>Stn9</i></b>	I	First dorsal spine	13	0.73	0.84	0.075*	56	
<b><i>Stn14</i></b>	I	-	21	0.78	0.90	0.073*	56	
<b><i>Stn16</i></b>	II	-	5	0.50	0.48	0.029	60	
<b><i>Stn23</i></b>	II	-	16	0.76	0.82	0.074*	56	
<b><i>Stn26</i></b>	II	First dorsal spine	13	0.79	0.82	0.054*	60	
<b><i>Stn30</i></b>	III	-	18	0.77	0.79	0.058*	60	
<b><i>Stn31</i></b>	III	-	14	0.84	0.88	0.041	60	
<b><i>Stn34</i></b>	III	-	16	0.72	0.83	0.097*	60	
<b><i>Stn38</i></b>	IV	-	2	0.21	0.22	0.025	60	
<b><i>Stn61</i></b>	VI	-	18	0.75	0.86	0.101*	62	
<b><i>Stn65</i></b>	VI	-	30	0.75	0.94	0.173*	52	
<b><i>Stn67</i></b>	VI	-	27	0.83	0.89	0.012	60	
<b><i>Stn69</i></b>	VII	-	13	0.78	0.84	0.118*	60	
<b><i>Stn70</i></b>	VII	-	7	0.71	0.73	0.074	60	
<b><i>Stn78</i></b>	VII	-	27	0.76	0.91	0.115*	60	
<b><i>Stn82</i></b>	VII	-	17	0.73	0.73	0.049	60	
<b><i>Stn83</i></b>	VIII	-	10	0.68	0.70	0.029	56	
<b><i>Stn93</i></b>	VIII	-	7	0.52	0.53	-0.006	62	
<b><i>Stn96</i></b>	VIII	Second dorsal spine	19	0.75	0.83	0.062	62	
<b><i>Stn98</i></b>	VIII	-	16	0.72	0.88	0.100*	56	
<b><i>Stn107</i></b>	IX	-	10	0.77	0.66	-0.051*	60	
<b><i>Stn130</i></b>	XI	Second dorsal spine	14	0.85	0.83	0.023	52	

<i>Stn131</i>	XI	Number of gill rakers	18	0.69	0.78	0.098*	52
<i>Stn134</i>	XII	-	4	0.36	0.50	0.310*	56
<b><i>Stn147</i></b>	XII	-	11	0.62	0.70	0.052	60
<i>Stn152</i>	XIII	Number of lateral plates	27	0.75	0.95	0.158*	52
<i>Stn158</i>	XIII	-	6	0.46	0.45	0.001	62
<b><i>Stn177</i></b>	XV	-	12	0.75	0.76	0.044	62
<i>Stn209</i>	XXVI	Number of lateral plates	2	0.20	0.22	0.090	62

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\*  $P \leq 0.05$  after Bonferroni correction.

**TABLE 3. Categorical model analysis of the stability of the direction of effects, positive or negative, of heterozygosity (response variable) in relation to fitness components and age cohorts (independent variables).** Complete saturated model in a), main effects model in b).

a)	df	$\chi^2$	P
Intercept	1	13.54	< 0.001
Cohort	1	0.07	0.785
Fitness component	3	12.23	0.007
Cohort * F. component	3	1.43	0.698
Likelihood ratio	0	-	-

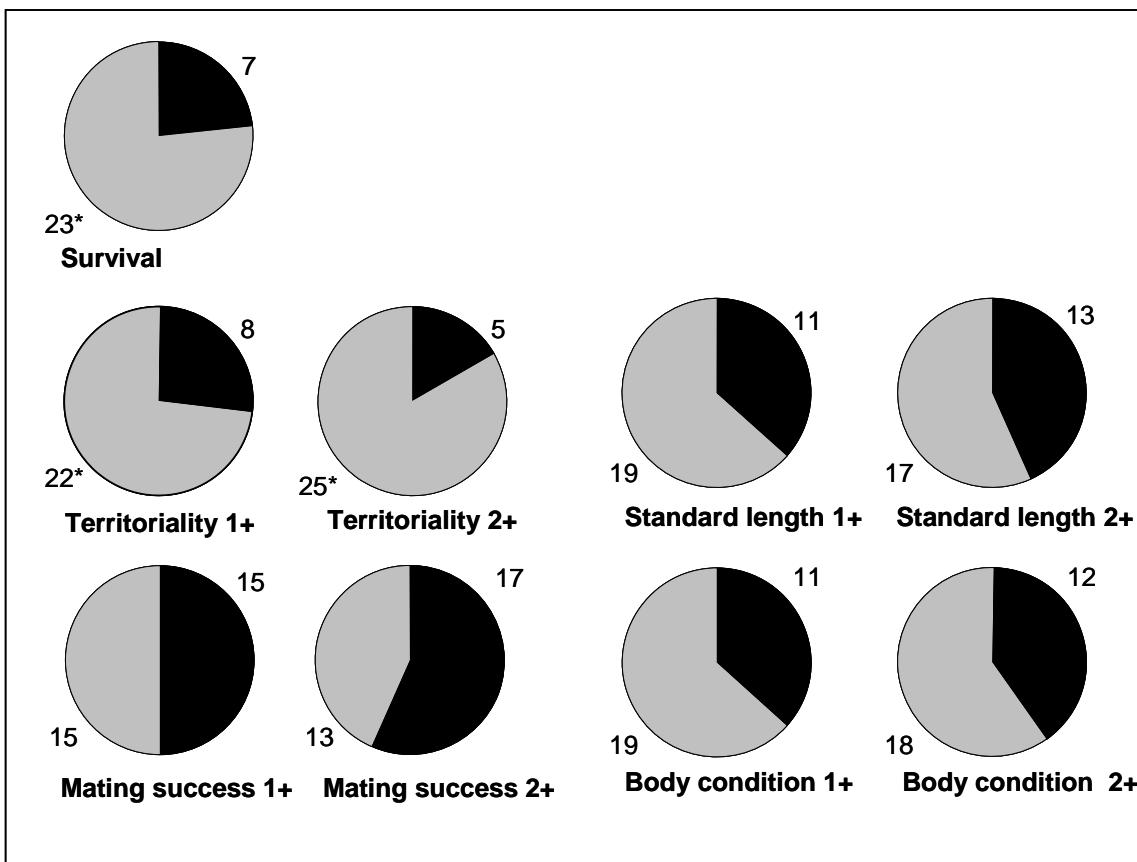
b)	df	$\chi^2$	P
Intercept	1	13.54	< 0.001
Cohort	1	0.07	0.785
Fitness component	3	12.23	0.007
Likelihood ratio	3	1.43	0.698

**TABLE 4.** Fisher's method combining probabilities from the single-locus tests, both for positive and negative HFCs. Loci with equal values for either positive or negative effects are omitted. The number of single-locus tests for combined probability is given in parentheses. Values of MLH-fitness coefficients of determination are presented only for significant ( $P \leq 0.05$ ) or nearly significant ( $P \leq 0.10$ ) tests. Significant probabilities ( $P \leq 0.05$ ) are shown in bold characters.

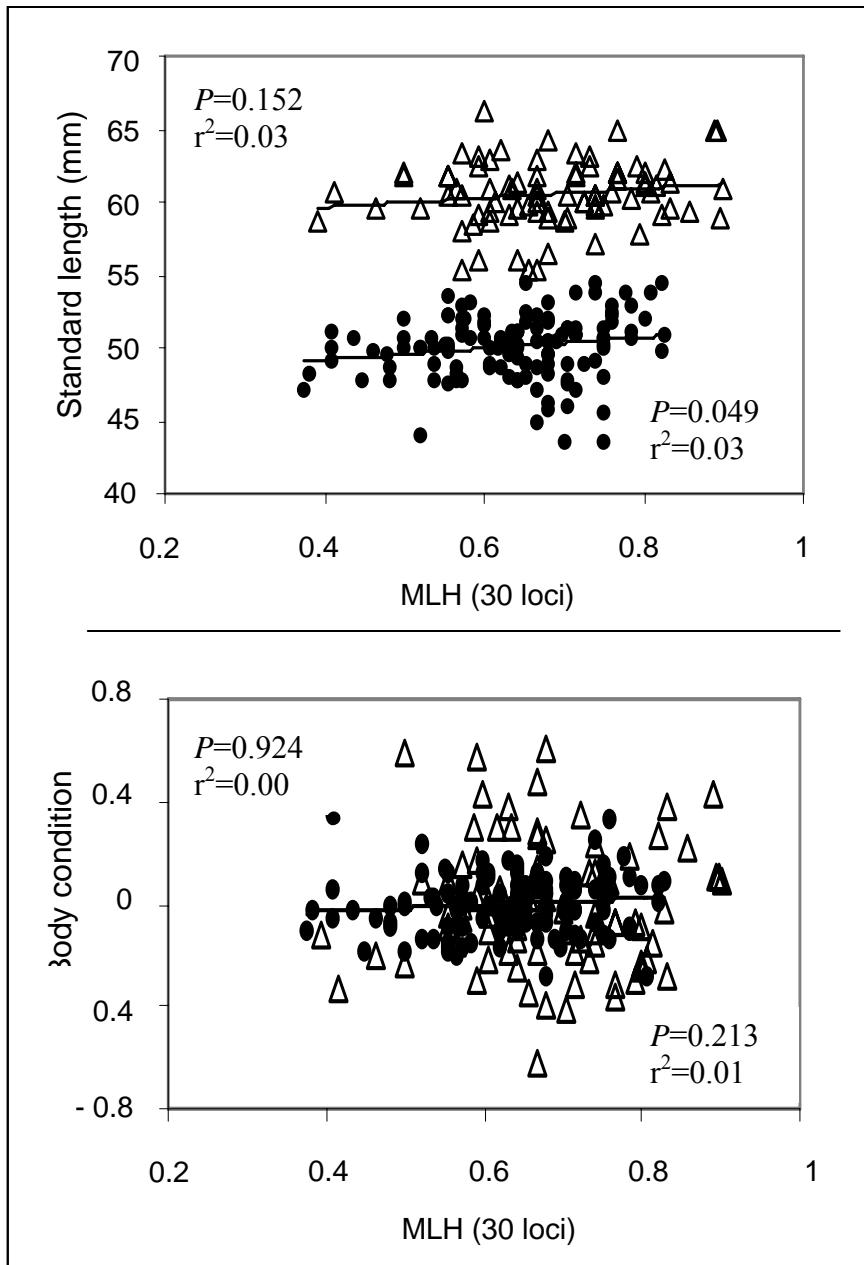
	Positive associations		Negative associations	
	$P$ (df)	$r^2$	$P$ (df)	$r^2$
Survival	0.039 (21)	0.06	0.816 (5)	-
Territoriality:	1+	0.073 (19)	0.08	0.428 (7)
	2+	<b>0.003</b> (18)	0.16	0.206 (4)
Mating success	1+	0.180 (15)	0.15	<b>0.017</b> (12)
	2+	<b>0.004</b> (11)	0.19	<b>0.000</b> (13)
Standard length	1+	0.066 (19)	0.12	0.849 (11)
	2+	<b>0.036</b> (17)	0.13	0.659 (13)
Body condition	1+	<b>0.037</b> (19)	0.08	0.588 (11)
	2+	0.495 (18)	-	0.256 (12)

**TABLE 5.** Selection differentials ( $S$ ) and standard errors calculated using MLH (multi-locus heterozygosity) estimate based on all 30 loci, those showing positive HFC (+ restricted MLH), or those showing negative HFC (- restricted MLH) in the case of mating success. Values that are not presented were all near zero. The number of loci used to estimate restricted MLH is provided in parentheses. Significant HFCs ( $P \leq 0.05$ ) are shown in bold characters.

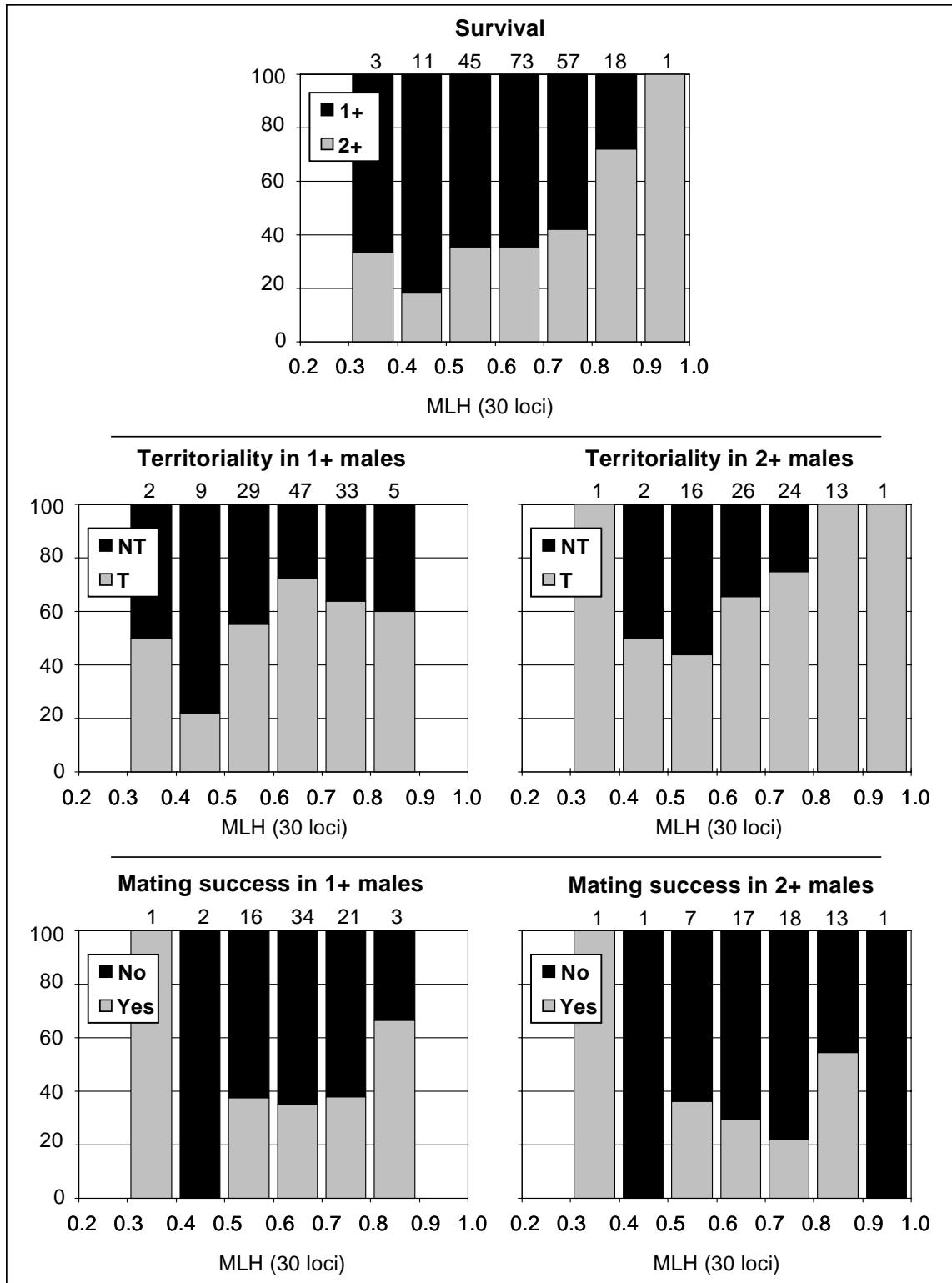
		$S$ , 30 loci MLH	$S$ , + restricted MLH (number of + loci)	$S$ , - restricted MLH (number of - loci)
Survival		$0.26 \pm 0.09$	$0.36 \pm 0.09$ (21)	-
Territoriality	1+	$0.15 \pm 0.07$	$0.25 \pm 0.07$ (19)	-
	2+	$0.21 \pm 0.08$	$0.26 \pm 0.07$ (18)	-
Mating success	1+	$0.02 \pm 0.15$	$0.56 \pm 0.17$ (15)	$-0.46 \pm 0.15$ (12)
	2+	$-0.12 \pm 0.17$	$0.64 \pm 0.20$ (11)	$-0.59 \pm 0.17$ (13)
Standard length	1+	$0.01 \pm 0.00$	$0.02 \pm 0.00$ (19)	-
	2+	$0.01 \pm 0.00$	$0.01 \pm 0.00$ (17)	-
Body condition	1+	$0.01 \pm 0.01$	$0.03 \pm 0.01$ (19)	-
	2+	$0.00 \pm 0.03$	$0.07 \pm 0.03$ (18)	-
Mean		0.06	0.24	-0.53



**FIGURE 1.** Numbers of positive and negative single-locus HFCs for each fitness component and cohort. Grey and black shading represent positive and negative HFCs, respectively. Significant differences in proportions, as estimated by cumulative binomial probability tests ( $P \leq 0.05$ ), are indicated by an asterisk.



**FIGURE 2.** Relationships between MLH (multi-locus heterozygosity) calculated from 30 loci and, from top to bottom, standard length and body condition for each age class. Males of the 2+ cohort are represented by open triangles, while 1+ males are represented by solid circles.  $P$ -values and regressions coefficients ( $r^2$ ) of the linear regressions are indicated in the bottom-right corners for the relationships obtained with 1+ males, and upper-left corners for relationships with 2+ individuals. The slope of the linear relationship is presented given a significant correlation or trend, as suggested by the data.



**FIGURE 3.** Relationships between MLH (multi-locus heterozygosity) calculated from 30 loci and, from top to bottom, survival, and territoriality and mating success for

**males of both age groups.** MLH (30 loci) is divided into distinct intervals to facilitate visualization of the relationships. The Y axis presents the percentage of males by category in each interval. NT and T refer to territorial and non-territorial males, respectively. Regarding mating success, the categories refer to nesting males with (Yes) or without (No) eggs in their nests. The number of individuals per interval is indicated at the top of each bar.

# **Chapitre 3**

## **Conclusion générale**

## Conclusion

Conserver la diversité génétique est un objectif primordial en conservation de la biodiversité, pour elle-même, mais aussi pour le potentiel évolutif qu'elle représente (Secretariat of the Convention on Biological Diversity 2001; Primack 2002). Conséquemment, le maintien de la diversité génétique est une considération primaire dans les programmes de conservation, entre autre, dans la reproduction assistée (e.g., Hedrick 2001; Wedekind 2002; Fiumera *et al.* 2004). Cependant, l'importance de l'hétérozygotie, en terme d'ampleur et de portée, fait toujours l'objet de litiges (e.g., David 1998; Hansson and Westerberg 2002). Entre autre, puisque les relations entre l'hétérozygotie et la fitness sont généralement faibles dans les populations naturelles (David 1998; Hansson and Westerberg 2002). Toutefois, la force des relations hétérozygotie-fitness a généralement été évaluée en corrélant l'hétérozygotie individuelle moyenne à des marqueurs génétiques à une ou des composantes de la fitness. Or, les travaux récents de Balloux *et al.* (2004) et de Slate *et al.* (2004) indiquent qu'il est probablement inadéquat d'utiliser l'hétérozygotie moyenne dans la plupart des populations.

En effet, la plupart des études emploient l'hétérozygotie moyenne aux marqueurs comme variable sous la prémissse que celle-ci représenterait l'hétérozygotie globale du génome, elle-même reflétant le niveau de consanguinité de l'individu (Balloux *et al.* 2004; Pemberton 2004; Slate *et al.* 2004). Or, ce serait le cas uniquement dans les populations hautement structurées ou avec reproductions consanguines fréquentes (Balloux *et al.* 2004). À la lumière de ces travaux, il est plus que pertinent de se demander si l'apparente faiblesse de la relation entre l'hétérozygotie et la fitness reflète une réalité biologique ou plutôt une approche méthodologique inadéquate.

Afin d'apporter un éclairage à cette question, nous avons conduit une étude sur la corrélation hétérozygotie-fitness (CHF) au sein d'une population sauvage d'épinches à trois épines. Cette étude a comme particularité de documenter la CHF à chacun des marqueurs (effets locaux) en plus de celle obtenue avec l'hétérozygotie moyenne (effet global). De plus, un nombre important de marqueurs génétiques (30) positionnés sur une carte de liaison (Peichel *et al.* 2001) a été employé, de même que cinq composantes de la

fitness (survie, succès d'appariement, territorialité, taille et condition physique). Spécifiquement, cette étude a permis de tester l'occurrence d'effets locaux de l'hétérozygotie à 30 marqueurs microsatellites sur cinq composantes de la fitness chez des mâles épinoches 1+ et 2+, d'évaluer l'importance de l'impact sur la fitness de ces CHF locales et de comparer les résultats obtenus par l'analyse des effets locaux aux résultats obtenus par une analyse de type effet global.

L'étude a révélé la présence d'effets locaux de l'hétérozygotie substantiels. Ces effets étaient présents pour toutes les composantes de la fitness. De plus, ils étaient vraisemblablement consistants d'une cohorte à l'autre. Les corrélations avec la fitness étaient importantes; elles expliquaient jusqu'à 22% de la variance du trait de fitness. Des différentiels de sélection déterminants ont également été détectés dans le cas de la survie, de la territorialité et du succès d'appariement. L'hétérozygotie à chacun des marqueurs avait généralement un effet positif sur la fitness. Cependant, des effets négatifs importants ont également été détectés dans le cas du succès d'appariement. Ainsi, les mâles les plus souvent choisis par les femelles étaient significativement plus fréquemment hétérozygotes à certains marqueurs, mais plus homozygotes à d'autres. De plus, à certains marqueurs, l'hétérozygotie n'avait apparemment pas d'effet sur les traits de fitness étudiés.

À l'opposé, les relations entre l'hétérozygotie moyenne et la fitness (effet global) étaient généralement faibles. Elles expliquaient seulement 5 % de la variance dans les traits de fitness en moyenne. De plus, aucune relation significative ou tendance n'a pu être détectée entre l'hétérozygotie moyenne aux 30 marqueurs microsatellites et la condition physique ou le succès d'appariement. Les relations obtenues avec l'hétérozygotie moyenne (effet global) étaient donc clairement plus faibles que celles détectées au niveau de l'hétérozygotie à chacun des marqueurs (effets locaux). Ces résultats viennent tout à fait appuyer les travaux de Balloux *et al.* (2004) et de Slate *et al.* (2004). Les effets locaux de l'hétérozygotie peuvent ainsi être sous-estimés au niveau global, probablement parce que l'effet de l'hétérozygotie sur la fitness à chaque marqueur peut être positif, négatif ou nul. Additionner ces différents effets serait donc propice à diminuer les différents signaux.

Cette étude a permis de démontrer que : (i) l'hétérozygotie moyenne n'est pas une variable adéquate pour évaluer la CHF dans les populations peu consanguines, (ii) l'hétérozygotie peut avoir des impacts majeurs sur la fitness même dans les populations non-consanguines, toutefois, ces effets se font vraisemblablement sentir au niveau local (à des gènes liés aux marqueurs) plutôt qu'au niveau global (à l'entièreté du génome). En plus de ces travaux, plusieurs autres études ont trouvé des évidences d'effets locaux de l'hétérozygotie (Bean *et al.* 2004; Hansson *et al.* 2004; Markert *et al.* 2004; Spielman *et al.* 2004; Syed and Chen 2005). À notre avis, les effets locaux de l'hétérozygotie sont plus répandus et plus importants que présumés jusqu'à maintenant. Nous croyons que les évidences devraient s'accumuler à mesure que s'effectueront des études appropriées considérant les CHF au niveau local.

Plusieurs impacts évolutifs peuvent résulter d'effets locaux de l'hétérozygotie. Ceux-ci ont été détaillés dans le chapitre deux. En résumé : les CHF locales positives pourraient contribuer à maintenir la diversité génétique aux gènes qu'elles affectent, à favoriser l'évolution et le maintien de comportements de dispersion et affecter le choix du partenaire et les autres phénomènes de sélection sexuelle. À ce sujet, dans la population étudiée, il est plausible que les CHF contribuent à favoriser le maintien de la variance génétique additive et non-additive par un comportement de choix de partenaire favorisant les mâles hétérozygotes à certains gènes.

Dans les futures études, il sera très intéressant de raffiner la compréhension et l'évaluation des relations entre l'hétérozygotie et la fitness en travaillant directement avec les gènes et les allèles effectifs, et en utilisant des estimateurs de la fitness plus raffinés (tel que le succès reproducteur à vie). De plus, il serait intéressant d'appliquer une méthode similaire pour étudier le phénomène de la vigueur hybride. Aussi, de façon plus générale, l'étude conjointe des aspects génétiques additifs et non-additifs participera certainement à une meilleure compréhension des phénomènes évolutifs.

Finalement, l'étude réalisée apporte un soutien aux décisions de conservation qui visent le maintien de la diversité génétique. Ainsi, la diversité génétique au sein des individus semble très importante même dans les populations peu affectées par la

consanguinité. Il reste toutefois à déterminer comment exactement ces effets au niveau de la fitness de l'individu se traduisent en effets sur la viabilité de la population. À tout le moins, les effets sur la survie des individus ont fort probablement des répercussions sur le maintien des populations.

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