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This version of Scientifur is the second issue of volume 27. After being published electronically, the first and the second issue of this volume will soon appear in a paper version to be sent to all our subscribers.

This issue contains a number of abstracts on various fur animal subjects including the abstract of the dissertation by Heli Lindeberg on *Embryo technology in the farmed European polecatt* (*Mustela putorius*) as well as the abstract of the dissertation by Anne-Mari Mustonen on *Seasonality, photoperiod and nutritional status in the control of endocrinological weight-regulation*. We are very pleased that we have been permitted to publicize these interesting dissertations, and we hope that our readers will provide us with more dissertations as well as articles for reviewing, proceedings, short communications, letters, etc.

On behalf of the
Group of Editors

Birthe Damgaard
VIII International Scientific Congress in Fur Animal Production

The Netherlands, 15 – 18 September, 2004

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Mapping of 53 loci in American mink (*Mustela vison*)

S.B. Kuznetsov, N.M. Mateeva, W.J. Murphy, S.J. O’Brien, O.L. Serov

Fifty-three genes were mapped in the American mink genome using polymerase chain reaction (PCR)-based analysis of a Chinese hamster–American mink somatic cell hybrid panel. Heterologous primers designed for cat gene mapping were used in this study. Forty-nine of these loci were localized into expected chromosome regions according to Zoo-FISH data, whereas four loci—ALPL, CDC20, ERF-2, and Fc(Mv)23617—were mapped out of expected conserved regions. PCR products amplified with primers corresponding to these four markers were partly sequenced and verified using BLAST. The results showed the homology to be more than 90% between mink and human or cat counterparts. At present, the gene map of American mink has expanded to 127 loci.

*Journal of Heredity, 2003: 94, 386-391, 1 fig, 3 tables, 22 refs.*

Is the western population of the European mink (*Mustela lutreola*) a distinct Management Unit for conservation?

J.R. Michaux, R. Libois, A. Davison, P. Chevret, R. Rosoux

The European mink (*Mustela lutreola*) is one of the most threatened carnivores in Europe, with fragmented populations in Belarus, Russia and Romania, as well in south-western France and northern Spain. Many populations have become extinct recently, or are declining. We investigated mitochondrial DNA variation, using the complete D-loop region, and concentrating on the west European population. The aim was two-fold: to use the genetic information to advise on the conservation of European mink, and to begin to understand their history through the Pleistocene. Captive breeding and re-introduction programmes are underway, so it is particularly vital to know whether the West European population should be treated separately. We find that European mink probably colonised from a single refugium after the last glaciation. West European populations may be fixed for a single haplotype, also suggesting a common origin. Despite this evidence for gene flow, following the precautionary principle we suggest that mink from the three geographically separate populations (Romania, Eastern and Western Europe) should be managed separately, for the moment.

*Biological Conservation, 2004: 115, 357-367, 4 figs, 3 tables, 55 refs.*

Variability of thirteen microsatellite markers in American mink (*Mustela vison*)

I.R. Vincent, A. Farid, C.J. Otieno

A mink genomic library was screened with an (AC)$_{15}$ oligonucleotide as the probe. Thirteen microsatellite loci were identified and primer sequences and amplification conditions were determined. All the 13 loci were polymorphic in black, brown, pastel and wild mink (*n* = 86), generating between 4 to 12 alleles per locus. Six of the primer pairs revealed polymorphisms in American pine marten.


Effects of hCG on folliculogenesis and fecundity in mink (*Mustela vison Schreb*)

D.V. Klotchkov, P.A. Eryuchenkov

The endogenous hormonal response obtained after reproductive organs are challenged by exogenous hormones is increasingly being used to predict presence of functional reserves and to apply this information to improve efficiency of managed breeding programs. With that in mind, the aim of the present study was to investigate the effect of a single treatment with hCG on folliculogenesis and fertility in standard 7-month-old mink females. The extent of stimulation following treatment was determined by examining patterns of vaginal smears. Characteristics of each cycle stage were: estrus, preponderance of cornified epithelial cells; proestrus, polygonal, elongate epithelial cells; anestrus, parabasal, intermediate and leucocyte cells. Smears exhibiting a mixed population of cells
were categorized as being in transition between adjacent stages anestrus–proestrus or proestrus–estrus. The initial evaluations were done on Day 6 after hCG treatment. Histomorphometric examination of ovaries and uteri was done during seasonal anestrus (November) and in the breeding season (March). Vaginal cytology patterns were correlated with changes in folliculogenesis. A mean of 1.3 mature (Graafian) follicles were counted during estrus, while the mean number seen during anestrus, anestrus–proestrus and proestrus, were 0.4, 0.3 and 1.0, respectively. During the breeding season, in females that were not treated, the numbers of growing follicles decreased and maturing follicles increased, whereas females that came in estrus after treatment with hCG in November had increased numbers of both growing and maturing follicles. Fertility after breeding in hCG-treated females was increased by 9.2% (P<0.05) as compared to untreated females. Females showing the highest fertility rise (27%) were predominantly in the group that showed estrus after hCG treatment. We conclude that monitoring the response of the mink reproductive system to hCG stimulation in November may be a useful tool for identifying females of high fertility in the spring.

Theriogenology, 2003: 60, 1583-1593, 1 fig, 3 tables, 23 refs.

**Evaluation of feed protein quantity by measuring plasma free amino acids in Atlantic salmon (Salmo salar L.) after dorsal aorta cannulation**


Two successive experiments were conducted in order to assess plasma free amino acid (FAA) profiles as a method for evaluating protein quality of fish feeds for Atlantic salmon, Salmo salar (L.). In experiment 1, the importance of meal size and inter-fish variation was assessed by using dorsal aorta cannulated fish and diets that contained different sources of fishmeal (menhaden versus herring) which in the case of herring, had been dried at either 70 or 100 °C. In experiment 2, an attempt was made to mimic a production situation by comparing the FAA profiles in salmon fed diets containing two commercially available fishmeals that had been produced in accordance with industrial standards (Norse-LT94® and NorSeaMink®; Norsildmel AL, Fyllingsdalen, Norway). FAA profiles in plasma 6 h after feeding were compared with feed true protein digestibility as determined in mink. Cannulated fish, held in individual tanks, were hand-fed twice daily to pellet rejection (satiety) and daily records of the actual rations consumed were maintained. A total of 24 different amino acids and other amino-containing compounds were detected using high-pressure liquid chromatography. Morning and evening meal size showed significant correlations. Meal size had a significant effect on blood levels of the majority of essential free amino acids (EAA) as well as the total sum of FAA (TFAA). In experiment 1, a marked inter-individual effect was found, possibly because of incipient sexual maturation. FAA profiles were therefore corrected for meal size by linear regression while repeated sampling via the permanently implanted cannula allowed paired comparisons of the different test diets, minimizing inter-individual variation. Significant differences in plasma FAA profile, EAA and TFAA were detected between fish fed all diets in both experiments. The preceding parameters for fish ingesting each feed were directly related to their respective mink protein digestibility in experiment 1, but not in experiment 2. Our results show that dietary protein quality can be differentiated by the aforementioned protocol, and by using fish with a low metabolic rate feed qualities could be ranked correctly as in experiment 1.


**Detection and sequence analysis of Danish and Swedish strains of mink astrovirus**


The sequences of mink astroviruses collected from 11 farms in Denmark and Sweden were analyzed and found to be homologous with one another but different from those of other astroviruses. A species-specific reverse transcriptase-PCR for mink astrovirus was established and shown to be suitable for the analysis of clinical samples.

Effects of temporal variation in the risk of predation by least weasel (*Mustela nivalis*) on feeding behaviour of field vole (*Microtus agrestis*)

E. Koivisto, J. Pusenius

Predation risk tends to vary in time. Thus prey animals face a problem of allocating feeding and antipredator effort across different risk situations. A recent model of Lima and Bednekoff (1999) predicts that a prey should allocate more feeding effort to low risk situations and more antipredator effort to high risk situations with increasing relative degree of risk in high risk situations (attack ratio). Furthermore when the proportion of time the prey spends in the high risk situation \(p\) increases, the prey have to eventually feed also in the high risk situations. However the increase in feeding effort in low risk situations should clearly exceed that in high risk situations as \(p\) increases. To test these predictions we measured feeding effort of field voles (*Microtus agrestis*) exposed to varying presence of least weasel (*Mustela nivalis*) and its feces in laboratory conditions. We generated quantitative predictions by estimating attack ratios from results of a pilot experiment. The model explained 15% of the observed variation in feeding effort of voles. Further analyses indicated that feeding effort was lower in high risk situations than in low risk situations at high attack ratio, but not at a lower one. Voles exposed to a presence of a weasel for extended periods showed signs of nutritional stress. Still we did not find any increase in feeding effort with increasing \(p\). This was obviously due to the relatively low maximal \(p\) we used as we included only conditions likely to occur in nature.

*Evolutionary Ecology, 2003: 17, 477-489, 4 figs, 2 tables, 25 refs.*

The effects of diisopropylmethylphosphonate on female mink: how medical intervention biased mortality data

E.J. Calabrese

This analysis retrospectively assessed the likelihood that medical intervention masked effects of diisopropylmethylphosphonate (DIMP) on female mink. Bucci et al. [Two-generation reproductive study in mink fed DIMP. Final report. Study no. TP-001. Prepared by Pathology Associates International, Jefferson, AR, USA, 1997] medically intervened in 24 out of 174 female mink to prevent mortality after an overdose of anesthetic was believed to have induced a life-threatening stress-syndrome. Bucci et al. [loc. cit.] dismissed the biological significance of the intervention since intervention occurred similarly in controls and treatment groups. The present investigation revealed that more (3-fold) DIMP-treated female mink surviving the intervention had abnormal physiological/clinical parameters at the time of intervention (9/10) than control female mink (3/9) (\(P<0.1\)). In addition, the severity of the physiological/clinical abnormalities increased with dose. These findings indicate that the DIMP treated female mink were more likely to have benefited from the intervention than the controls. The intervention, therefore, reduced the likelihood of observing treatment-related effects.

*Regulatory Toxicology and Pharmacology, 2003: 38, 260-268, 1 fig, 7 tables, 4 refs.*

Breeding suppression in free-ranging grey-sided voles under the influence of predator odour

O. Fuelling, S. Halle

The breeding suppression hypothesis predicts that females of certain small mammal species will reduce reproduction as a response to the odour of a specialised mammalian predator. This was tested in a field experiment with grey-sided voles (*Clethrionomys rufocanus*) during three summer seasons (1997–1999) in the subalpine tundra of northern Norway, which is a natural habitat of this species. In a first phase free-ranging voles in six unfenced 1-ha plots were monitored by live-trapping from June to August each year. In a second phase from August to September, three of the plots were sprayed with weasel (*Mustela nivalis*) odour to simulate increased apparent predation risk, while the remaining three plots served as untreated controls. On all plots voles were individually marked with ear tattoos and were regularly live-trapped during the whole breeding season to follow their performance. On the treatment plots the recruitment rate of juveniles did not increase in late summer as it did on the control plots. The proportion of reproductively non-active adult females was significantly higher on
the treatment plots for both old and young females. Our results thus verify the breeding suppression hypothesis for the first time under natural conditions. However, the response in overwintered females is in conflict with the original hypothesis because the assumed fitness benefits from breeding delayed until the next season are inaccessible to them. As an alternative explanation we propose a short-term response of reduced activity and interrupted breeding until the predator has exploited and left the feeding patch. Such a “duck and cover” strategy would increase the fitness of females of all age classes when prey habitats are patchy.


Multiphasic growth curves in mink (Mustela vison) selected for feed efficiency

K. Sørensen, M. Grossman, W.J. Koops

Growth of mink from 1 to 28 weeks of age was modelled, using weekly body weights, and effects on model parameters of selection lines for high and low feed efficiency, sex and interaction were tested. From results on average growth curves, individual growth curves were modelled using a multiphasic logistic growth function with three phases. Individual growth curve parameters were tested for effects of selection line, sex, and interaction by analysis of variance.

Selection for feed efficiency affected the growth curve of mink. Selection lines differed for time of maximum increase (center) for each phase; the high feed efficiency line was centered later than the low line, the growth curve being shifted to the right. Males gained more weight in phases 2 and 3 than females; phase 2 was centered later for males than for females; males had a shorter duration for phase 3 than females. There was no line-sex interaction.


Size allometry in mink (Mustela vison) selected for feed efficiency

K. Sørensen, W.J. Koops, M. Grossman

Objectives were to analyse absolute and relative size of mink at maturity, and to test effects of selection line, sex and interaction on size. For male and female mink selected for high or low feed efficiency, size at 30 weeks was analyzed for body weight, carcass weight, pelt weight, subcutaneous fat weight, and pelt length. For absolute size, an ANOVA model included effects of line, sex, and interaction. For relative size, two models were used: an allometric model and an extended allometric model, which included effects of line, sex, and interaction.

For the ANOVA model, sexes differed for each variable; females were less than males. As a percentage of body weight, however, carcass weight was larger in females than males, whereas fat weight was smaller in females than males. For the extended allometric growth model, sexes differed for carcass weight and subcutaneous fat weight; females fattened faster than males.


Effects of carbohydrate-free diets on the performance of lactating mink (Mustela vison) and the growth performance of suckling kits

B.M. Damgaard, C.F. Børsting, K.L. Ingvartsen, R. Fink

The effects of carbohydrate-free diets on body weight changes, feed consumption, and plasma concentrations of nutrients, metabolites, and hormones in lactating mink and on growth performance in kits were investigated in a total of 108 litters during the lactation period of seven weeks. The litters were distributed in three experimental groups fed different ratios of metabolisable energy (ME) derived from protein and fat (61:38; 47:52; 33:66). The females and kits were weighed at parturition and 2, 3, 4, and 7 weeks after parturition. Blood samples were collected from females in the second, fourth, and sixth week of the lactation period. Carbohydrate-free diets fed to
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Lactating mink can in principle support normal performance, health of the females, and growth performance of the kits. However, mink need a sufficient supply of available gluconeogenic precursors in the form of amino acids. In the present experiment, the diet with 33% of ME from protein contained insufficient amounts of gluconeogenic precursors for lactating mink, and three weeks post partum this group was excluded from the experiment. The differences in dietary protein level (61% and 47% of ME) and fat level (38% and 52% of ME) influenced the plasma concentrations of free fatty acids (FFA) and urea, but had minimal influence on plasma concentrations of other nutrients, metabolites, and hormones.


Effects of high dietary levels of fresh or oxidised fish oil on performance and blood parameters in female mink (Mustela vison) during the winter, reproduction, lactation and early growth periods

B.M. Damgaard, C.F. Børsting, R.M.Engberg, S.K. Jensen

The effects of high dietary levels of fresh or moderately oxidised fish oil on performance and blood parameters in mink females were investigated during the winter, reproduction, and lactation periods. Furthermore, the effects of the diets on kit performance were investigated during the lactation and early growth periods. The investigation was carried out with a total of 292 females distributed in five experimental groups fed fresh fish oil stored frozen, fresh fish oil ensiled, oxidised fish oil stored frozen, oxidised fish oil ensiled, and soya oil, respectively. The females were weighed three times during the winter period, and the females and the kits were weighed at parturition and 2, 4, and 7 weeks post partum. Blood samples were collected from the females and the kits 6 and 8 weeks post partum, respectively. The results show that high dietary levels of fresh or moderately oxidised fish oil could be used for mink females during the winter and reproduction periods without any negative effects on performance, health and reproduction results. However, high levels of fish oil resulted in lower kit weights at weaning. These negative effects on kit growth were related to the dietary composition fed during the lactation and early growth periods and not to the diet used during the preceding winter and pregnancy periods. A high intake of polyunsaturated fatty acids resulted in a decreased number of blood platelets for both mink females and their kits.


Assessing animal welfare in a strictly synchronous production system: the mink case

S.H. Møller, S.W. Hansen, J.T. Sørensen

Most on-farm welfare assessment systems have been developed for use in dairy and pig farms. These production systems are non-synchronous, in the sense that the same processes occur continuously throughout the year. Animal welfare during most or all phases of production may therefore be assessed at any time of the year, except for some effects of season. Many domesticated farm animals such as sheep, goats, deer and mink are seasonally synchronised in their production, in the same way as were their wild ancestors. A comprehensive welfare assessment system including animal-based indicators for these species must therefore take an entire production cycle into consideration. This can be illustrated by a welfare assessment protocol developed and tested by the Danish Institute of Agricultural Sciences (DIAS) for mink production. The DIAS concept is based on indicators from four sources: the system, the system’s management, animal behaviour, and animal health. An advantage of seasonality is that the measurement of welfare indicators can be optimised and standardised in terms of age/season and sample size, making reliable results relatively cheap to obtain. Furthermore, there is ample time to plan the requisite interventions. A disadvantage of seasonality is that the entire herd may have been at risk when a welfare problem is disclosed by direct animal-based indicators; for example, the entire herd may have been exposed to a social grouping causing bite marks, which can be observed at pelting. Based on observation of the social grouping, this can be corrected before fighting and biting occurs. Based on observation of the bite marks, corrections are postponed until next season. Welfare assessment intended for decision support in a synchronous
production system should therefore include a higher proportion of early indicators based on the system and management, in order to prevent the development of potential welfare problems involving the entire herd. The assessment of animal-based indicators may be relatively cheap and more reliable in synchronous production compared to non-synchronous production, and these indicators are therefore given high priority as they reflect the welfare resulting from the corrections made based on indirect system and management indicators.

*Animal Welfare, 2003: 12, 699-703, 1 fig, 1 table, 8 refs.*

**In-cage sandbox as a ground substitute for farmed blue foxes (Alopex lagopus): Effects on digging activity and welfare**

**H.T. Korhonen, L. Jauhiainen, T. Rekilä**

A study on the behavioural and welfare effects of in-cage sandboxes was carried out on juvenile farm-bred blue foxes (*Alopex lagopus*) with special reference to digging behaviours and time spent on sand substrate. Twelve juvenile male blue foxes were used in each of two experimental groups: (1) a test group and (2) a control group. Animals were raised singly in cages measuring 120 cm long x 105 cm wide x 70 cm high, from weaning in July to pelting in December. All experimental animals were housed conventionally but cages of the test group contained in-cage sandboxes (80 cm long x 40 cm wide x 14 cm high). Various physiological, behavioural, health and production-related variables were measured during the study. Final body weights of test animals were significantly (*P* = 0.05) lower than controls. Occurrence of endoparasites (*Toxascaris leonine, Isospora* sp.) did not substantially differ between groups. Open field activity was greater (*P* = 0.02) and latency to touch novel objects shorter (*P* = 0.02) in the test group compared with the control. Cortisol-creatinine ratio, incidence of stereotypes, size of adrenals or other organs, blood screen and fur quality parameters were not significantly different. Sandbox hygiene deteriorated rapidly during the experimental period. Fur coats of test animals were dirtier than those of controls only in October. Altogether nine different sandbox behaviours were observed in the test foxes. Digging was the fifth most common behaviour, comprising 5.8% of total sandbox use. Amount of time spent in the sandbox peaked in July, averaging 117 min/24 h, and declined towards winter. The most common sandbox behaviours observed were walking (24.3% of total time), sitting (22.0%) and resting (17.5%). Results indicated low motivation to use in-cage sandboxes as a digging substrate. On the other hand, the presence of in-cage sandboxes may provide opportunities for foxes to engage in other species-specific activities and/or seek sensory comfort through contact with the sand. The effects of in-cage sandboxes on animal welfare need further study.

Embryo technology in the farmed European polecat (*Mustela putorius*)

Doctoral dissertation

by

Heli Lindeberg

Institute of Applied Biotechnology
University of Kuopio
2003

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The present research project started the development of assisted reproductive technology in endangered mammals in Finland. It concentrated on the endangered European mink (*Mustela lutreola*) which is assumed to have become extinct in Finland. The farmed European polecat (*Mustela putorius*) served as a model animal for the European mink. Studies on embryo technology of the farmed European polecat focused on the development of embryo recovery, cryopreservation and transfer techniques, which could further be applied to the conservation of the endangered European mink.

Oestrous donors were mated to fertile males once daily on two consecutive days. The recipients were mated to vasectomized males to induce ovulation. A total of 582 embryos were recovered from 66 donors either post mortem or surgically under anaesthesia 3 to 13 days after the first mating. The embryos were subjected to one of the following treatments 1) fresh embryo transfer to recipients 2) in vitro culture 3) conventional slow freezing, thawing and transfer to recipients 4) vitrification, warming, in vitro culture and transfer to recipients.

During in vitro culture, 1- to 16-cell stage embryos developed to blastocysts but did not expand. The embryos placed in culture as morulae or blastocysts expanded in vitro during the first 24-h period in the same manner as their in vivo counterparts. In vitro
culture of polecat embryos using the described technology is applicable for temporary storage of embryos waiting to be transferred or cryopreserved.

The numbers of transferred embryos per recipient were 10.8, 10.0 and 12.5 for fresh, frozen-thawed and vitrified-warmed embryos, respectively. The percentages of live kits per transferred embryos (= survival rate) were 42, 11 and 16, and the numbers of live kits per recipient were 4.5, 1.1 and 2.0 for fresh, frozen-thawed and vitrified-warmed embryos, respectively, with an overall survival rate of 30% (89 live kits/302 transferred embryos). Transfer of cryopreserved embryos resulted in a low kit yield, but nonetheless did produce the first mustelids ever from frozen-thawed and vitrified-warmed embryos.

Universal Decimal Classification: 619, 502.74, 636.93, 591.16

CAB Thesaurus: Mustelidae; polecats; embryo transfer; cryopreservation; in vitro culture; embryonic development
Seasonality, photoperiod and nutritional status in the control of endocrinological weight-regulation

Academic dissertation
by
Anne-Mari Mustonen

Department of Biology
University of Joensuu
2003

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Leptin and ghrelin are novel weight-regulatory peptides whose functions have been mainly studied in laboratory rodents and humans. Exogenous leptin decreases their appetite and body mass, whereas ghrelin is an orexigenic hormone stimulating food intake and fat gain. The darkness hormone melatonin times seasonal rhythms and controls energy metabolism of vertebrates. As these weight-regulatory hormones have evolved to improve the adaptations of animals to their changing environments, it is important to investigate their functions and interactions also in wild animals.

The aim of this thesis was to investigate the effects of season, light and nutritional status on the endocrinological weight-regulation of vertebrates. The possible interactions of leptin and ghrelin with the seasonal rhythms of body weight, fat content, appetite and reproduction were studied in the raccoon dog (Nyctereutes procyonoides) and the burbot (Lota lota) exhibiting excessive but nonpathological accumulation of fat in their adipose tissue and liver. The characteristics of the endocrine response to fasting of the raccoon dog were determined during a two-month winter sleep induced by fasting. The effects of a lack or an excess of melatonin on weight-regulation were investigated in the seasonal raccoon dog and the nonseasonal laboratory rat (Rattus norvegicus). The autumnal leptin and growth hormone concentrations of the raccoon dog plasma were low but increasing, whereas their ghrelin levels were high, probably inducing hyperphagia and fat gain. The high leptin and growth hormone concentrations together with the low ghrelin levels during the mid-winter could have induced lipolysis, protein conservation and sleepiness. Long-term wintertime fasting decreased the plasma insulin levels and the activities of the thyroid gland and the adrenal cortex, enabling lipid mobilization and efficient protein sparing. Melatonin treatment advanced the seasonal rhythms of leptin, ghrelin and growth hormone, whereas the fasting-induced winter sleep did not affect their concentrations.

Exogenous melatonin or continuous light had no influence on the plasma leptin or growth hormone concentrations of the nonseasonal rat, but melatonin decreased their ghrelin concentrations. Constant illumination stimulated the carbohydrate
metabolism of the rat liver, whereas melatonin elevated the utilization of liver carbohydrates but suppressed the mobilization of fat. Both constant light and exogenous melatonin stimulated the renal utilization of carbohydrates. The levels of leptin- and ghrelin-like immunoreactivities in the burbot plasma increased after spawning. The burbot liver contained high concentrations of leptin-like immunoreactivity, suggesting that it could be one production site for the leptin-like peptide in teleost fish.

Keywords: ghrelin, growth hormone, leptin, lipid metabolism, Lota lota, melatonin, nitrogen metabolism, Nyctereutes procyonoides, Rattus norvegicus, seasonality, weight-regulation, winter sleep
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