# Caucasian Black Grouse Research, Monitoring and Conservation Management in Georgia

## Deliverable # 1:

# Data Collation and Identification of Study Areas

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## Submitted to:

Baku-Tbilisi-Ceyhan (BTC) Pipeline and Southern Caucasus Pipeline (SCP) operated by BP

May 2004, Tbilisi, Georgia

# Content

# Introduction

The Caucasian Black Grouse Research, Monitoring and Conservation Management project in Georgia is part of the Environmental Investment Program (EIP) that is initiated and funded by BP and its partner companies in Baku-Tbilisi-Ceyhan (BTC) oil export pipeline and South Caucasus (SCP) gas pipeline projects. Georgian Center for the Conservation of Wildlife (GCCW) implements project in partnership to BirdLife International (European Division Office), University of Idaho (Fish and Wildlife Department) and World Pheasant Association (WPA).

The project goals are to provide the scientific basis for the effective conservation management of the endemic and rare faunal species that is of global conservation concern - Caucasian Black Grouse, and to create capacity to perform such management in Georgia.

One of the objectives of the project is to collate available data and identify areas for intensive ecological and population studies in Georgia from published and other sources on species distribution, habitat requirements and other information relevant to its conservation. To achieve this objective, activity is conducted during January-April, 2004, and present document describes its results.

Chapter one summarizes information on existing data and status of Caucasian Black Grouse focusing on the following topics: Conservation status and current state of knowledge; Distribution and evolutionary relationships; Main habitats and ecology; Population size and trends.

Chapter two outlines required information for the species effective conservation management.

Chapter three describes planned research activities and provides following information for each study component: Applied methods; Sites selection; Needed human resources; Needed equipment; and Expected results.

Document also provides full bibliography of Caucasian Black Grouse and a map of known sites of species presence in Georgia.

**Acknowledgments.** The following persons have actively participated in compilation of this document, in its review and in provision of useful comments and suggestions: David Tarkhnishvili, Ramaz Gokhelashvili, Lexo Gavashelishvili, Zurab Javakhishvili, Phil McGowan, Ilse Storch, Kerry Reese, Cort Anderson, Szabolcs Nagy, Mike J. McGrady, Peter Capainolo, James Dean, Rene Dekker and Roald Potapov. We are grateful of their assistance and support.

# 1. Existing Data and Status of Caucasian Black Grouse

The existing data of the CBG biology, ecology and distribution was compiled by GCCW in 1998-2002. Largely, this information is given in Gokhelashvili et al. (2003), but some later publications and our field observations allow us to update this information.

## *1.1. Conservation status and current state of knowledge*

The Caucasian Black Grouse (*Tetrao mlokosiewiczi*) is classified as a species of global conservation concern that is endemic to the Caucasus region. It is one of the grouse species with the smallest distribution and most highly fragmented range. The species is listed in the national Red Data Books of all range countries as rare with declining populations, and is included in several international listings: Species of European Concern, category 2 with status "Insufficiently Known"; as a Restricted-range Species; and in the 2002 IUCN Red List of Animals as "Data Deficient". This IUCN category means that the species is too poorly known for its threat status to be properly judged. IUCN states that these species should be afforded the same attention as threatened species until their status is clarified. This is one of two grouse species that are of high global conservation concern, the other being the recently described Gunnison Sage-grouse (*Centrocercus minimus*), from North America, which is listed as Endangered on the IUCN Red List. It is also listed as one of the highest priorities for action in the IUCN/WPA Grouse Specialist Group Action Plan (Storch 2004). Due to its small range, the difficulty of access to its remote, high mountain habitat, and its relatively small population, it is a poorly studied species.

## *1.2. Distribution and evolutionary relationships*

CBG is found at both the northern and the southern slopes of the Great Caucasus mountain range, between basins of the rivers Belaya and Samur, and along the ranges of the Lesser Caucasus, including an extreme northwestern Iran, Zangezur, Karabakh, Pambak-Sevan, Bazum, Javakheti, Trialeti, Meskheti, Erusheti, Shavsheti, and eastern Karadeniz (Ponto) mountains. Species total range approximates 12,000 $km^2$ and covers 6 countries: Russia, Georgia, Azerbaijan, Armenia, Turkey and Iran. About 50% of the range is in Georgia. In general, the range of the species coincides with the distribution of forested mountains of the western and central Caucasus with large enough areas elevated above 2000 m a.s.l.

Information on the CBG findings in Georgia (both from the bibliography and our observations) is compiled and the locations are given in the GIS-based map (Figure 1).

Attachment 2 provides information on Caucasian Black Grouse specimens from different museums and data holdings of the world. WPA has compiled this information and continues to update it.

The range of CBG is completely isolated from the range of its closest relative – European Black Grouse (*Tetrao tetrix*) - that is widespread throughout forests of central and eastern Europe and western Siberia. Recent molecular genetic analyses by Lucchini et al. (2001) show that the split between the ancestors of CBG and European Black Grouse happened in

the early glaciation period, over a million years ago, as a result of fragmentation of mountain forest areas in the Ice Age. Fossils of Caucasian Black Grouse are found from the Upper Pleistocene (Burchak-Abramovich 1974, Janossy 1976, Potapov 1985). In contrast to the European Black Grouse during the Holocene (10-15 thousands of years before present) spread from the Alps throughout a huge area of western Eurasia, the CBG has never spread beyond the area of its origin in the Caucasus Isthmus.

### *1.3. Main habitats and ecology*

CBG is found at the upper timberline, between sub-alpine forests and sub-alpine meadows, at the elevation between ca. 1,300 and 3,000 m. a.s.l. (most common between 1,500 and 2,500 m a.s.l.). Winter habitats lie in the upper part of the mountain forest belt, between 1500-1800 m. a.s.l., whereas summer habitats are mainly in the sub-alpine belt, above 1800-1900 m a.s.l. An important feature of the CBG summer habitats appears to be the presence of shrubby areas that provide necessary shelters for nesting birds. These shrubs throughout the large part of the range are represented by the Caucasian Alpine Rose (*Rhododendron caucasicum*), but occasionally other shrubs (e.g. yellow azalea or juniper) can be used as well.

Open south-facing grassy slopes are used as lekking sites [do the BP people know what a lek is, or should thie be explained?]. Typically, the same areas are used year after year. Birds appear at leks in early April and lekking males can be observed throughout June, although individual displaying males can be seen throughout the entire warm season.

Nesting begins in May or early June. The male does not participate in reproduction after fertilization. Reported clutch sizes differ considerably between 2-13, average 6.0.

The incubation period has been estimated indirectly as 20-25 days. Young start flying at 10-14 days and are reach size of adults at about 2 months. Females became sexually mature in their first year, but males probably do not breed until at least 2 years old.

More detailed information on the breeding ecology, habitat dynamics, feeding habits, and behavior of the CBG can be found in Gokhelashvili et al. (2003) and in papers listed in the Attachment 1.

### *1.4. Population size and trends*

No precise information on the population size of CBG in Georgia is available. The range of the bird appears to be separated in at least five fragments; the Lesser Caucasus range is more fragmented than the range in the Great Caucasus (Figure 1). In optimal habitats of the Great Caucasus the density appears to be as twice as high that in the Lesser Caucasus, however since standardized method were never applied for exact population estimates the question about population density remains open. According to very rough extrapolative estimate, this may vary between 40,000 – 60,000 birds.

## 2. Information Required for an Effective Conservation Management

In order to outline the optimal strategy for the CBG conservation in Georgia, the following information is necessary:

- The exact distribution of CBG within the country and the "potential range" of CBG that shows to what extent the ability of the bird to occupy its habitats is realized. This allows us to monitor the entire national population.

- The location of areas with the highest concentration of CBG habitats. Such areas serve as "population sources" and maintain not only the populations of CBG they have but also populations in the adjacent areas, if migration between the locations is not prevented by natural or man-made barriers.

- The potential of migration between the individual localities, that shows an ability of the birds to repopulate the areas where CBG is extinct by different reasons, reflected in the existing gene flow.

- At least rough estimate of the population size and spatial distribution of the density

- The individual range of males and females and seasonal dynamics of habitat use.

We also need to know what pressures there are on the species so that we can estimate their impact on wild populations of the CBG. This also allows us to monitor both the scale of the pressure (e.g. livestock grazing) and the actual populations as well. Therefore we can change management accordingly.

The existing information on the CBG distribution, ecology, and population size is too fragmented to provide precise enough answers on these questions. The planned research is aimed to fill in gaps of the knowledge and to provide exhaustive information associated with the enumerated problems.

# 3. Description of Planned Research Activities

The anticipated study includes three main blocks, differing with applied approaches and methodologies, field techniques, equipment, and analytical tools. The description of each of these methodological blocks is given below.

### *3.1. Population Biology Study*

This includes the analysis of individual ranges of males of females, localization of nesting sites, description of the seasonal habitat dynamics, and estimation of demographic characteristics of CBG in the selected sites. The radio tracking method will be applied to study population biology aspects of Caucasian Black Grouse in 2 sites. About 30 individuals at each study site will be marked, in order to determine reproductive effort, reproductive success, habitats used for nesting, brooding, and wintering, number of chicks per female recruited to the fall population, to establish seasonal movements and ranges of the birds and to estimate survival rates.

***Applied methods.*** In the preliminary research plan, several potential methods of catching birds were outlined, including capturing birds with drop-nets, walk-in funnel traps or mist-nets on leks during spring, and by funnel traps during summer and early fall (Caizergues and Ellison, 1998). As a result of the discussion between the fieldwork team members and Dr. Kerry Reese, that followed the study of the grouse leking site near Kazbegi, walk-in funnel traps were decided to be the most appropriate method. The method was repeatedly and successfully applied for the spruce grouse in the western US, and the habitats of the Caucasian Black Grouse appear to be quite appropriate for the application of this method.

Radio telemetry has never been applied for the Caucasian Black Grouse study. Taking into account the difficulty of accessing CBG habitats, only 20 individuals will be radio-mark during the first season (10 in each study area). An additional 40 birds will be radio-marked in the second season (20 in each study area). Each hen will receive a 10.5 gm radio transmitter attached to a necklace. This radio package is < 1.5% of the minimal weight (712 gm) of a female Caucasian Black Grouse (Madge and McGowan, 2002). Each bird will be released at the point of capture.

Radio-equipped grouse will be monitored at least once per week from release through August using a portable receiver and hand-held antenna. Birds will be approached to within 50-60 m depending on terrain and vegetation cover and circled using the loudest signal method of Springer (1979). Bird locations will be obtained using a Garmin Etrex 12 Channel GPS Satellite navigation unit and cover type will be recorded for each individual grouse location, and at each nest site and brood site. Radios on mortality signal will be located as quickly as possible in attempts to define the cause of mortality (predator, accident, human caused, starvation). The same data will be recorded for all non-radio marked grouse incidentally observed in the course of routine fieldwork. Care will be taken to avoid disturbing grouse, but when birds do flush the number, sex and ages will be noted when possible.

Nest sites and their immediate vicinity will be characterized by cover type, percentage and heights of shrubs and understory vegetation, and visual obstruction measurements (Connelly et al. 2003). To minimize researcher disturbance to incubating hens, clutch size will be determined from the number of eggs in successful nests after the brood leaves the nest bowl, or eggs will be counted at nests if the female is off the nest on an incubation break. Nests will be considered successful if 1 or more eggs hatch. Percentage of hens nesting, clutch size, egg fertility, nest success, re-nesting effort and success, and hen success will be calculated from these data.

Brood hens will be monitored 1-2 times weekly from hatch through August. Cover of vegetation types and GPS locations of brood will be obtained. Initial size of each brood will be determined from the number of eggs hatched, and the final brood size will be counted by flushing young at 6 to 7 weeks of age before broods begin mixing in larger groups. This will provide a crude estimate of brood survival and number of chicks per female recruited to the fall population, information necessary for the population viability analysis.

From September to March, radio-marked grouse will be located once per month to monitor seasonal movements, seasonal habitat use, seasonal timing of mortality, and estimate seasonal and annual survival. These data will also help define the spatial range of the species by season using ArcView v.3.3 software package, and be components in the population viability analysis. An ArcView Extension, Animal Movement (Hooge and Eichenlaub 1997) will be used to estimate home range sizes of individual hens. Survival analysis will be conducted in program MARK (White and Burnham 1999), which incorporates the Kaplan-Meier product limit method with staggered entry (Pollock et al. 1989) so that new hens can be added to the radio-marked sample throughout the spring and summer.

***Site Selection.*** The following criteria were applied to select two sites for the population biology study: Site protection status and human pressure must be different (one should be protected area and the other non-protected); Geographical distance between sites must be as large as possible; Population dynamics of the sites must be practically independent; Feasibility of conducting study at these sites must be higher than in other sites (easier access, higher bird density, etc); and Safety of conducting work. Based on these criteria the following two sites were selected: Lagodekhi reserve in the eastern Great Caucasus and Shavsheti mountain range in the western Lesser Caucasus (Akhaltsikhe district).

***Needed Human Resources.*** The local team of at least three persons is necessary at each site. A Georgian bird expert coordinates study that includes training of local team members, testing of equipment and coordination of fieldwork. Consultations from an international grouse expert is required in developing study plan and methodology, in identifying and purchasing the radio equipment;, in providing training to the Georgian bird expert and in data analyses.

***Needed Equipment & Consumables.*** The following equipment is needed to conduct CBG population biology study:

1. Radio transmitters (50 units – you say 60 above), Model A3950, battery 2343, 35 pulses per minute, mortality switch, battery life of 262-524 days, 26 cm (round), 14 cm wide, a

disk rather than a cylinder, weight: 13.2 gm (1.65% of hen weight), Frequency range 150.000-150.999 MHz.

2. Receivers (3 units): The Field Master (FM100) (durable, inexpensive, simple and portable receiver). Weight of each receiver 0.6 kg, 10 cm x 16 cm x 5.5 cm and runs from 6 AA batteries for 15 hours of continuous use. Frequency range 150.000-150.999 MHz

3. 3-element folding Yagi antennas (3 units). Frequency range 150-154 MHz;

4. Extra coaxial cables 1.5 meters long (2 units)

5. Headsets (3 units)

6. Transmitters with extra material to make necklace

7. Extra batteries for receiver (AA batteries)

8. Two photo-cameras with extra batteries

9. Four binoculars

10. Two telescope with tripods

11. Two sets of camping equipment (tents, sleeping bags, mats, cords, gas heater, etc)

12. Six walk-in funnel traps. For preparation should be used steel net with the mesh diameter 5 cm (for constructing fences between the traps, 3 m each) and a flexible metal net with the mesh diameter ca. 3 cm for constructing the traps themselves

13. Two GPS units with spare batteries (Garmin Etrex 12 Channel GPS Satellite)

14. Two units of the following materials: Spring scale to weigh the birds; Cotton weighing bag; Screw driver needed to change the batteries in the receiver; Wing band (ladies nylon stocking to hold bird in); Side-cutting wire cutters to snip transmitter cable; Crimp pliers (any size pliers will do); Scissors; Small needlenose pliers; Data sheets with extra pencils, pens and a clipboard; Plastic bag to put data sheets and clipboard in if it rains/snows

***Expected results*.** The clear answers on the following questions are expected as a result of the population study in the two selected sites: (1) where exactly are located: lekking sites, nesting areas, summer, autumn, and winter habitats of the CBG; (2) seasonal dynamics of the habitats; (3) species reproductive potential in the selected sites; (4) key limiting factors.

### *3.2. Current Distribution and Habitat Requirements Study*

The preliminary plan of the study of current distribution of CBG and development of the stochastic distribution model based on the habitat requirement study was adjusted during the first phase of the Project. This was done during the international CBG meeting in Ankara, Turkey (March 18-23 2004) and in discussions between the all research team members.

The range reconstruction will be done in two steps. The analysis of published data allowed us to outline an approximate range of the species in Georgia (Fig. 2 – there is no Fig 2 in my version of the document). This baseline data will be supplemented by additional field study in two areas where presence of grouse remains under question: Samsari range and Javakheti range in southern Georgia. Establishment of grouse presence in larger geographic regions will be followed by more detailed description of exact locations where the bird is found. It is planned to develop a probability model of the CBG distribution, which shows not just potential range of the bird, but also the density of the appropriate habitats in individual areas based on the 1:200,000 scale topographic map.

***Applied methods.*** Each location will be mapped using GPS a point theme [I do not know what a 'point theme' is] of grouse presence/absence will be created in GIS. Each location will be briefly described: the vegetation type, the elevation, exposition, presence and density of sheep or cattle herd, location of nearest settlement and occasional additional variables will be scored locally. After preparing the detailed distribution map, a set of environmental variables, including landscape, vegetation type, hilliness, steepness, and exposition, will be scored from 1:200,000 scale digitized topographic maps of Georgia, with five GIS layers. Analyses of the factors will be performed using ArcView v.3.3 software package (ESRI, Redlands, CA)

Values from the set of factors will be extracted for each point in the point theme of grouse occurrence using Extract Zvalues script. The resultant table in the dBASE format will be transferred into SPSS v. 11 for Windows (SPSS Inc., Chicago, IL) to perform binomial (or binary) logistic regression. This approach is used when the dependent variable is dichotomous (in this case, 1 and 0: Grouse presence and absence, respectively) and the independent variables are of any type (Hosmer, et al 1989; Menard, 2002). Logistic regression estimates parameters (coefficients) after transforming the dependent variable into a logit variable: $\mathbf{Ln}[p/(1-p)]$, where **Ln** is the natural logarithm and $p$ is the probability of obtaining a positive response (in our case grouse presence).

Unlike ordinary least squares (OLS) regression that calculates changes in the dependent variable itself, logistic regression calculates changes in the logit variable:

$\mathbf{Ln}[p/(1-p)] = \mathbf{B_0} + \mathbf{B_1X_1} + \mathbf{B_2X_2} + \ldots..,$ where $\mathbf{B_0}, \mathbf{B_1}, \mathbf{B_2}, \ldots$ are parameters to be estimated from the observed data and $\mathbf{X_1}, \mathbf{X_2}, \ldots$are the independent (i.e. explanatory) variables. Logistic regression seeks to maximize the log likelihood (**LL**) which reflects how likely it is that the observed values of the dependent variable may be predicted from the observed values of the independent variables. **LL** varies from 0 to minus infinity.

A pseudo $\mathbf{R^2}$ statistic (e.g. Nagelkerke's **R**-Square) is available to summarize the strength of the relationship. The success of the model is assessed by checking a classification table for correct versus incorrect classifications of the dichotomous dependent variable. The Wald statistic is used to test the significance of individual independent variables. It equals the ratio of **B** to **SE** squared, where **B** is a parameter estimate and **SE** is its standard error.

Running the Forward Stepwise Likelihood Ratio and Enter methods, the model that explains most of the **LL**, classifies the data best, and whose parameter estimates are statistically significant (**$p<0.05$**) will be chosen.

To finalize the model, residual analysis will be performed obtaining: (1) A scatter plot of Standardized residuals against the independent variables, which will be examined to see if the independent variables in the model are linearly related to the logit of the dependent variable. If the scatter plot shows a pattern (i.e. a certain shape of relationship), the relationship between the logit and an independent variable may not be linear. Quadratic, cubic, square root, logarithmic and inverse transformations will be tested to eliminate nonlinearity. (2) Scatter plots of leverage values (a measure of how much a case influences the regression) and Cook's distances (a measure of how much the coefficients change when a case is removed from the model) will be examined in order to reveal influential points that are the result of errors in data collection or entry. Cases with leverage values > $2p/n$, where $p$ is the number of independent variables in the model, and $n$ is the number of cases will be examined more closely, as will be cases with Cook's distances > 1.

To evaluate the performance of the classification schemes, Cohen's kappa will be used as a measure of the agreement between the observed values and predicted group values. A value of 1 indicates perfect agreement, while that of 0 indicates that agreement is no better than chance.

***Site selection.*** The following criteria were applied to select the sites for this study: Maximum coverage of CBG potential range in Georgia; Effectiveness of time/man power (avoidance of duplications); Relatively easy access to the site; and Safety of conducting work. Following geographic areas were selected for the study: Great Caucasus - Lagodekhi area, Tusheti, Kazbegi area, and upper currents [does mean the upper stretch of a river?] of Rioni; Lesser Caucasus - western part of Trialeti range (Bakuriani-Kodiani area), Samsari range, Javakheti range, Meskheti range (Zekari area), and Erusheti range. 10 to 20 sites will be visited and described for each of the enumerated geographic regions.

***Needed Human Resources.*** A local person at each site is involved in the study. A Georgian bird expert coordinates study that includes training of local persons, coordination of fieldwork, and data analyses. A GIS operator is needed to transfer the obtained data in the GIS environment.

***Needed Equipment & Consumables.*** Field equipment necessary for this task includes general equipment (backpacks, slipping bags, tents etc.) and GPS devices.

The full list of the field equipment is given below:

1. A Photo-camera with extra batteries

2. Nine binoculars

3. A Telescope with tripod

4. Camping equipment (tents, sleeping bags, mats, cords, gas heater, etc)

5. A GPS unit with spare batteries (Garmin Etrex 12 Channel GPS Satellite)

6. 1:200,000 scale digitalized topographic maps of Georgia with all layers (relief, roads, settlements, river network, vegetation);

7. Satellite images of vegetation cover of Georgia;

8. GIS software ArcView v.3.3 software package (ESRI, Redlands, CA)

***Expected results.*** As a result of the study, the predicted map of the CBG distribution in GIS environment will be produced, with the exact positioning of optimal and sub-optimal potential habitats of the species throughout the Georgian part of its range. This will give us the necessary knowledge on the exact distribution of the species as well as on the areas that can serve as "population sources" and the conservation of which is of the highest priority for species survival.

### *3.3. Species Range Fragmentation Pattern and Connectivity Level Study*

This research block will provide an important piece of information about the structure of the CBG range, including: (1) the level and timing of historical isolation between separate geographic populations of CBG, in particular those from the Great and the Lesser Caucasus: it is important to know whther or not the Great Caucasian and the Lesser Caucasian populations represent independent evolutionary lineages (and since what time), in which population dynamics, population genetic structure, and evolution are fully separate; (2) the level of ongoing gene flow between the populations and how it depends on the geographic distance and natural barriers: this will help us to distinguish between self-sustainable populations from those which may strongly depend on the neighboring localities.

***Applied methods.*** In the course of the study, it is planned to amplify and study both mitochondrial and nuclear genes of CBG for further analysis with population genetic methods. The CBG feathers (collected in the sites selected for population and distribution studies) will be used to apply modern molecular genetic methods. Three major techniques will be used: (1) Analysis of mitochondrial DNA sequences in samples to estimate both the divergence between the regional populations of the Caucasian Black Grouse and to establish presence/absence of gene flow; (2) Scoring of genotypes at 5-6 polymorphic microsatellite loci for large samples (at least 15 individuals in each) to estimate the actual level of gene flow among populations; and (3) Applying existing simulation models (combining the population biology data, habitats requirements, distribution patterns and molecular genetic study results) to evaluate viability of individual populations and the entire national population, and to estimate the probability of local extinctions.

*Sampling*. Preliminary fieldwork conducted in April 2004 showed that feces of the birds (that can be used for sequencing of mitochondrial DNA fragments) are easy to collect; however, feathers are highly desirable to conduct full-scale genetic investigation (including microsatellite genotype scoring). Feces and feathers are stored dry in small polyethylene bags, and blood taken from captured birds will be stored in eppendorf tubes with a specialized buffer obtained from the University of Idaho. Large samples of feces from Kazbegi and Bakuriani area were already collected and transported to LECG (Moscow, Idaho; see below) for optimization of the DNA extraction and PCR methods.

DNA will be extracted from tissue samples following standard phenol-chlorophorm protocols (Sambrook et al., 1989), with optional modification of the method for poor-quality tissue samples (Hillis et al., 1995; Tan & Orrego, 1992; Vachot & Monnerot, 1996; Cano & Poinar, 1993) and stored in TE solution under +4°C (Sambrook et al., 1989).

*Sequencing of mitochondrial genes*. There are sequences of cytochrome b, mitochondrial control region, and some other mitochondrial genes of the Caucasian Black Grouse, available from the GenBank. Original primer sequences are reported in the publication of Lucchini et al. (2001). Because the mitochondrial control region (“D-loop”) is the most variable of known sequences, we plan to amplify and sequence this region from at least 5 individuals for each identified site. This will allow us to estimate the timing of separation and the level of genetic divergence between the populations from distant regions of the Caucasus (such as Greater versus Lesser Caucasus, Black Sea Basin versus Caspian Sea Basin, etc.) and to

determine which geographic populations can be considered as isolated genetic units. PCR - amplifications will be performed on thermal clyclers (MJ Research DNA Engine) available in the partner molecular laboratory (**L**aboratory for **E**cological and **C**onservation **G**enetics (**LECG**) at the University of Idaho, USA). Amplicons will be directly sequenced using the Big Dye v. 3.1 Labelling Kit (Applied Biosystems) and standard PCR primers, following manufacturer's protocol. Gel electrophoresis and visualisation of the sequencing products will be accomplished using the ABI 377 automated DNA sequencer (Applied Biosystems). Sequences obtained during the course of this study will be deposited in GenBank. Because reconstruction of phylogenies is not among the priorities, it will only be optionally used for resolving individual questions connected with the history of the distribution of the grouse in the Caucasus. If needed, parsimony and maximum likelihood methods will be applied (Felsenstein, 1985), using PAUP (Swofford, 2002). It will be also use nested-clade analysis to sort haplotypes. Estimation of the isolation time will be done, following outlines in Moritz et al. (1996) and Schmidt et al. (2000).

*Analysis of microsatellte genotypes*. In a recent paper, Piertney and Hoglund (2001) published primers appropriate for amplification of eight microsatellite loci of European [you have used tthis name earlier, and need to be consistent throughout the document] Black Grouse (*Tetrao tetrix).* Since European Black Grouse and Caucasian Black Grouse are closely related sister species (Lucchini et al., 2001), the same primers (or at least majority of them) should be applicable for amplifying microsatellite loci in the Caucasian Black Grouse. Five to six loci will be amplified by PCR for at least 15 individuals from each site, and visualized by acrylamide gel electrophoresis on an ABI 377 with multiple fluorescent-primer labelling. This will allow high through-put of samples. Gels will be scored using GeneScan and Genotyper Analysis software (ABI), and the resultant genetic data analyzed using a variety of programs, including: a) POPGENE (1997); b) FSTAT (Goudet, 1994); c) MIGRATE (Beerly, 2001); d) STRUCTURE (Pritchard et al., 2000).

*Estimation of viability of populations and selecting priorities.* As a result of fieldwork, a set of data will be obtained that can help to estimate viability of different populations. Those include information on the demography (basic information on the age structure – juveniles, yearlings, and adults, sex ratio, survival rates, productivity, brood survival), sample population size, approximate carrying capacity of the range and its individual portions, and dispersal rates between the range fragments estimated by genetic methods. This will help to model probability of extinction of isolated populations and the entire species, dependent on the extent and rates of the degradation of the habitats as a result of human pressure. In order to estimate viability of individual populations, the software VORTEX 9.14 (2003) will be applied. In order to estimate viability of metapopulation systems, including separate regional populations and the entire range, the software RAMAS 4.0 GIS/Metapop (2003) will be applied.

Based on the results of the modelling, the sites within the CBG range may be identified that need urgent and special attention, as opposed to portions of the range where extinction probability is relatively low. We will also estimate relative risk coming from different types of causes (including direct human impact such as hunting, habitat degradation, and accidental extinction due to habitat fragmentation). Such information is essential to develop monitoring and conservation management plans.

***Site selection.*** At least 15 individual samples will be collected from each of six selected regions: Lagodekhi, Kazbegi, Racha, Samsari (if presence of CBG there will be confirmed), Trialeti, and Meskheti-Erusheti. These sites represent (1) both the Greater and the Lesser Caucasian parts of the CBG range; (2) both the northern and the southern slopes of the Great Caucasus, and (3) most distant available parts of CBG at the both mountain systems.

***Needed Human Resources.*** Samples will be collected during population biology and distribution studies, so that no additional personnel are required for this task. A Wildlife Genetics expert is required to coordinate this study (samples collection, laboratory analyses, data analyses, training for a genetics assistant). A genetics assistant conducts laboratory work in the University of Idaho. Head of laboratory provides consultations in sampling, storing and laboratory analyses.

***Needed Equipment & Consumables.*** No special equipment for collecting tissue samples for the DNA analysis is required, but plastic bags for storing feathers and feces, syringes for taking blood from the captured birds, and small plastic containers with a specialized buffer obtained from the LECG for storing blood. Equipment and consumables for the molecular genetic study, as well as the software necessary for the analysis are available at LECG (and some analytical software is available at GCCW).

***Expected results.*** The spatial structure of CBG range will be clarified as a result of this research block. We will know, how many isolated geographic populations the CBG currently consists of. Moreover, we will know the intensity of gene flow between the distant areas of the species range, which is necessary for predicting the probability of extinction and re-population in individual localities and outlining “weak” areas where irreversible extinction is most probable and which may need artificial re-stocking.

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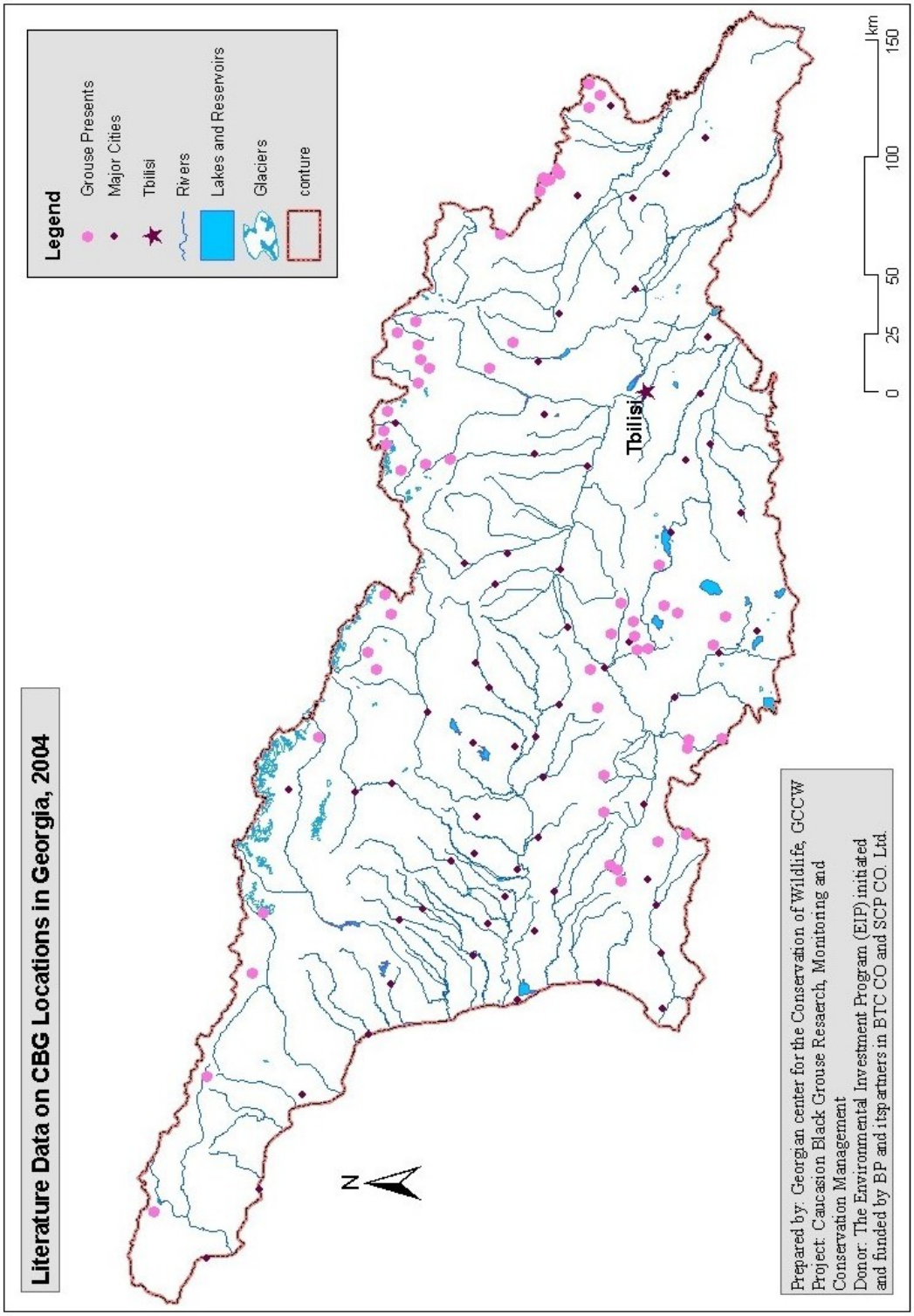
Figure 1. Known points of CBG distribution in Georgia



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## Attachment 2. Information on Caucasian Black Grouse from different data holdings (compiled by WPA)

### 1. American Museum of Natural History, USA. *From Peter Capainolo on 21st May 04*

| Catalogue number | Species | Sex | Location |
|---|---|---|---|
| 539930 (8) | *Tetrao mlokosiewiczi* | M | CAUCASUS, RUSSIA |
| 539929 (60) | *Tetrao mlokosiewiczi* | ? | CAUCASUS, RUSSIA |
| 539928 (62) | *Tetrao mlokosiewiczi* | ? | CAUCASUS, RUSSIA |
| 539927 (61) | *Tetrao mlokosiewiczi* | ? | CAUCASUS, RUSSIA |
| 539926 (158) | *Tetrao mlokosiewiczi* | M | SOURCES OF THE LABA, KUBAN OBLAST, CAUCASUS, RUSSIA |
| 539925 (163) | *Tetrao mlokosiewiczi* | ? | SOURCES OF THE LABA, KUBAN, OBLAST, CAUCASUS, RUSSIA |
| 539924 | *Tetrao mlokosiewiczi* | M | DERBENT, DAGESTON, CAUCASUS, RUSSIA |
| 539923 | *Tetrao mlokosiewiczi* | F | DERBENT, DAGESTON, CAUCASUS, RUSSIA |
| 539922 | *Tetrao mlokosiewiczi* | F | MT. ESCHKAKON, TEREK OBLAST, NORTH CAUCASUS, RUSSIA |
| 539921 | *Tetrao mlokosiewiczi* | F | MT. ESCHKAKON, TEREK OBLAST, NORTH CAUCASUS, RUSSIA |
| 539920 | *Tetrao mlokosiewiczi* | M | MT. ESCHKAKON, TEREK OBLAST, NORTH CAUCASUS, RUSSIA |
| 539919 | *Tetrao mlokosiewiczi* | M | MT.ESCHKAKON, TEREK OBLAST, NORTH CAUCASUS, RUSSIA |
| 539918 | *Tetrao mlokosiewiczi* | M | MT.BERMAMY, NORTH CAUCASUS, RUSSIA |
| 539917 | *Tetrao mlokosiewiczi* | M | MT.BERMAMY, NORTH CAUCASUS, RUSSIA |
| 539916 | *Tetrao mlokosiewiczi* | M | MUSCHT, TEREK OBLAST, NORTH CAUCASUS, RUSSIA |

### 2. Chicago Academy of Sciences, USA. *From online database May 04*

| | | | |
|---|---|---|---|
| No specimens | *Tetrao mlokosiewiczi* | | |

### 3. University of Connecticut Bird Collection, USA. *From online database May 04*

| Catalogue number | Species | Sex | Location | Date |
|---|---|---|---|---|
| ANSP Number 108601 | *Tetrao mlokosiewiczi* | | Caucasus Mountains, Russia | collected 2 November 1902 |

**4. Cornell University, USA.** From online database May 04

| | | | |
|---|---|---|---|
| No specimens | *Tetrao mlokosiewiczi* | | |

**5. Yale Peabody Museum, USA.** *From online database May 04*

| | | | |
|---|---|---|---|
| No specimens | *Tetrao mlokosiewiczi* | | |

**6. University of California, Berkeley, USA.** *From online database May 04*

| | | | |
|---|---|---|---|
| No specimens | *Tetrao mlokosiewiczi* | | |

**7. Academy of Natural Sciences, Philadelphia, USA.** *From online database May 04 and detail from Nate Rice 17 May 04*

| Catalogue number | Species | Sex | Location |
|---|---|---|---|
| ANSP Number 108601 | *Tetrao mlokosiewiczi* | | |

**8. Dept. of Ornithology and Mammalogy, California Academy of Sciences, USA**. From online database May 04

| Catalogue number | Species | Sex | Location | Date |
|---|---|---|---|---|
| CAS ORN 42803 | *Tetrao mlokosiewiczi* | | Prebai-Kuban Oblast, N Caucasus Mts. | 1892 |
| CAS ORN 42804 | *Tetrao mlokosiewiczi* | | Prebai-Kuban Oblast, N Caucasus Mts. | 1892 |

**9. The Field Museum, Chicago, USA.** From online database May 04

| Catalogue number | Species | Sex | Location | Date |
|---|---|---|---|---|
| FMNH#: 406926 | *Tetrao mlokosiewiczi* | | Tersche-Oblask, N Caucasus | 1895 |

**10. National Museum of Natural History, Smithsonian Institution, Washington, USA.** *From James Dean 18 May 04*

| Catalogue number | Species | Sex | Location | Date |
|---|---|---|---|---|
| | *Tetrao mlokosiewiczi* | | Kuban District, North Caucasus | 1 May 1892 |
| | *Tetrao mlokosiewiczi* | | Kuban District, North Caucasus | 1 May 1892 |
| | *Tetrao mlokosiewiczi* | | Caucasus Mts | 14 May 1894 |

**11. National Museum of National History, The Netherlands.** *From Rene Dekker 19 May 04*

| Catalogue number | Species | Sex | Location | Date | Notes |
|---|---|---|---|---|---|
| | *Tetrao mlokosiewiczi* | | Horda, Caucasus | 6 January 1898 | |
| | *Tetrao mlokosiewiczi* | | Horda, Caucasus | 6 January 1898 | |
| 14 specimens | *Tetrao mlokosiewiczi* | | Caucasus | Between 1898 and 1904 | Purchased from a dealer |

**12. The Natural History Museum, Tring, London.** *From visit 20 May 04*

| Catalogue number | Species | Sex | Location | Date | Notes |
|---|---|---|---|---|---|
| 1956.57.38 | *Tetrao mlokosiewiczi* | M | Central transcaucasus | 3 Oct 1935 | From Darwin State Museum, Moscow in 1956 |
| 1965.M.1670 | *Tetrao mlokosiewiczi* | M | Bermaccussiz, Taritz, North Caucasus | 17 Dec 1888 | Collector = Sushkin. Ex-Meinertzhagen collection |
| 89.11.2.59 | *Tetrao mlokosiewiczi* | M | Muschty, Ciscaucasia | 22 Feb 1886 | ex-A.P. Hoolst (or Koolst) collection |
| 81.5.1.5303 | *Tetrao mlokosiewiczi* | M | Caucasus | | ex-J Gould collection |
| 96.1.1.453 | *Tetrao mlokosiewiczi* | M | Wolfthor am Bermamit, North Caucasus | | ex P.Aug Holst coll, via Henry Seebohm |
| 75.11.4.1 | *Tetrao mlokosiewiczi* | M | Caucasus | 20 Jul 1892 | Presented by Count Branicki |
| 1965.M.1668 | *Tetrao mlokosiewiczi* | M im | Bermamud, North Caucasus | 4 Dec 1886 | Collector = Dronoz (or Dronow) and ex-Meinertzhagen |
| 1965.M.1669 | *Tetrao mlokosiewiczi* | F | Bermamud, North Caucasus | 2 Dec 1886 | Collector = Dronoz (or Dronow) and ex-Meinertzhagen |

| | | | | | |
|---|---|---|---|---|---|
| 96.1.1.453 | *Tetrao mlokosiewiczi* | F | Bermamit-Tersche, North Caucasis Oblast | | ex P.Aug Holst coll, via Henry Seebohm collection |
| 75.11.4.2 | *Tetrao mlokosiewiczi* | M | Caucasus | 20 Jul 1892 | Presented by Count Branicki |
| 1956.57.39 | *Tetrao mlokosiewiczi* | M imm | Tareck-Milasz, North Caucasus | Mar 1898 | Ex-Darwin State Museum, via Meinertzhagen |
| 87.11.2.59 | *Tetrao mlokosiewiczi* | F | Bermamud, Ciscausia | 4 Dec 1886 | ex-A.P. Hoolst (or Koolst) collection |
| 96.1.1.455 | *Tetrao mlokosiewiczi* | M juv | Eschkakon Mt, Near Bermamit, North Caucasus | | ex P.Aug Holst coll, via Henry Seebohm collection |
| 76.11.24.1 | *Tetrao mlokosiewiczi* | M | Caucasus | | Presented by Count Branicki |
| 1956.57.49 | *Tetrao mlokosiewiczi* | F | Eschkakon Mt, Terek-Oblast North Caucasus | 8 Mar 1890 | Ex- Darwin State Museum |
| 87.11.2.58 | *Tetrao mlokosiewiczi* | M | Ciscausia | 2 Dec 1896 | ex P.Aug Holst coll, via Henry Seebohm collection |
| 96.1.1.454 | *Tetrao mlokosiewiczi* | F | Bermamit, North Caucasus | | Seebohm bequest. Mounted specimen |
| 96.1.1.456 | *Tetrao mlokosiewiczi* | M imm | Bermamit, North Caucasus | | Seebohm bequest. Mounted specimen |
| 96.1.1.451 | *Tetrao mlokosiewiczi* | F | Tersche Oblast,Caucasus | | Seebohm bequest. Mounted specimen |

**13. Zoological Museum of the Zoological Institute of the Russian Academy of Sciences, St Petersburg.** *From Roald Potapov 21 May 04*

| Catalogue number | Species | Sex | Location | Date |
|---|---|---|---|---|
| 137626 | *Tetrao mlokosiewiczi* | M ad | Mount Kodiani, 20 km east from city Borjomi, approximately 41 Grade 37 minutes N - 43 grade 47 min.E. | 6.05.1957 |
| 137625 | *Tetrao mlokosiewiczi* | M s.ad. | The same place | 17.05.1957 |
| 137624 | *Tetrao mlokosiewiczi* | F ad | The same place | 14.09.1957 |
| 23836 | *Tetrao mlokosiewiczi* | M ad | Sources of the river Adjaris-Zhali, app.41 Grade 30 min.N - 42 Grade 30 min.E. | 27.12.1895 |
| 23955 | *Tetrao mlokosiewiczi* | F ad | The same place | 20.06.1893 |
| 23835 | *Tetrao mlokosiewiczi* | Male ad | The same place | 27.12.1895 |

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